INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

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IBO CHALLENGE II

32nd INTERNATIONAL BIOLOGY OLYMPIAD

Lisbon, PORTUGAL - July 21st, 2021

THEORETICAL TEST

(Time available: 180 minutes)

- This test consists of 249 questions arranged in 54 Groups (Q1- Q54).
- All questions have the same value – 1 point each correct answer.
- Maximum score: 249 points
- Only answers marked in the ANSWER SHEET will be validated

GENERAL INSTRUCTIONS

1. Your exam is composed of seven topics.

2. Your exam is composed of three types of questions:
   - Multiple choice questions
   - True / False questions
   - Matching items questions

3. The students are not supposed to use words to answer the questions, only codes of letters placed in the appropriate locations in the answer sheet.
   - Multiple choice questions should be answered by selecting the correct answer on the Answer Sheet
   - True / false questions should be answered by selecting either a "**T**" for true or an "**F**" for false, on the Answer Sheet
   - Matching items questions should be answered on the Answer Sheet by selecting the appropriate letter in the key provided in each case

ENJOY AND HAVE FUN!!!

THEORETICAL TEST

Topics:

I. Q1 - Q12 – Cell Biology
II. Q13 - Q21 – Plant Anatomy and Physiology
III. Q22 - Q33 – Animal Anatomy and Physiology
IV. Q34 - Q35 – Ethology
V. Q36 - Q45 – Genetics and Evolution
VI. Q46 - Q51 – Ecology
VII. Q52 - Q54 – Biosystematics

I. Cell Biology

Q1

To trace the path of a secretory protein from its synthesis to its export from a cell, suppose that you have added radioactive amino acids to a culture of cells, and then measured the amount of radioactivity that shows up in the proteins of each of the following cell fractions at different times after addition.

I – secretory vesicles
II – Golgi complex
III – rough ER
IV – nucleus

Mark as true (T) or false (F) the order in which the proteins of these fractions exhibit radioactivity (*4 points, 1 point each correct answer*)

A. III → II → IV → I → out of the cell
B. III → II → I → out of the cell
C. IV → III → II → I → out of the cell
D. IV → II → III → I → out of the cell

Q2

For each of the following phases of the cell cycle, indicate whether the chromosome number is haploid (H), diploid (D) or could be either (E). Also indicate whether chromosomes have replicate (R) or have not replicated (N/R) (Chromosome status). (*10 points, 1 point each correct answer*)

Chromosome number	Chromosome status	Cell cycle phase
	2	
		Meiotic metaphase I
		Mitotic prometaphase
		Meiotic interkinesis
	1	

Q3

Indicate whether each of the following statements is true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. Centrosomes replicate independently of chromosomes.

B. The nuclear envelope becomes fragmented at mitosis, and it is distributed between the daughter cells like other membrane-bounded organelles such as ER and Golgi

C. The total DNA content of a cell following meiosis is half that of a cell after mitosis.

D. Microtubule polymerization, depolymerization, and microtubule motor proteins are required for DNA replication.

Q4

Indicate whether each of the following statements is true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. Plasmolysis occurs when a plant tissue is placed in a hypotonic medium.

B. Animal cells will lyse when they are placed in distilled water.

C. When plant cells are placed in a hyperosmotic sucrose solution, sugar crosses the cell wall but not the cell membrane, keeping the cell the same size.

D. In plant cells water loss is normally a reversible phenomenon, while in animal cells it is not.

Q5

Observations in optical microscopy are often accompanied by the measurement of the specimen under study. Among other methodologies, the measurement can be performed after calibration, of each objective, with the aid of an eyepiece micrometer (ocular lens) and a microscope stage micrometer.

I. To calibrate an objective, it is necessary to start by placing the eyepiece micrometer (EM in Plate 1) in position on the microscope tube and installing the microscope stage micrometer (SM in Plate 1) on the microscope stage.

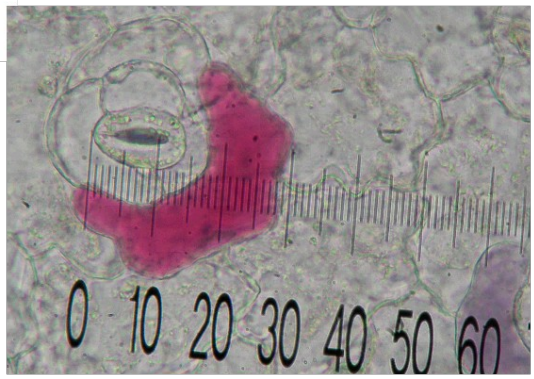
II. Then, the scales of the eyepiece micrometer and the microscope stage micrometer must be aligned, so that they are parallel, partially overlap, and so that the beginning of the graduation of the two scales

superimpose.

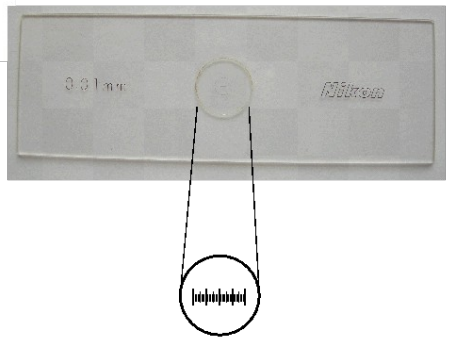
III. Having superimposed the zeros of the two scales, look for the point at which the two scales overlap equally.

IV. In the example of Figure C from Plate 1, 70 divisions of the eyepiece micrometer correspond exactly to 0.4 mm (= 400 µm) of the microscope stage micrometer. This value is only valid for the lens used.

A



B



C

Based on the description above, and Plate 1, **indicate whether each of the following statements is true (T) or false (F).** (*4 points, 1 point each correct answer*)

A. 1 ocular unit = 5.7 µm.

B. The procedure described in II is represented schematically in Figure B.

C. Figure B shows a microscope stage micrometer.

D. The stoma in Figure A is approximately 80 µm in length.

Q6

Cellular life depends on maintaining a balance between external and internal environments. Any change may have major consequences, so exchange is dynamic and moves in both directions across the cell membrane.

Mark with **true (T) or false (F),** the characteristics listed in the table below about membrane transport. (*8 points, 1 point each correct answer*)

	Features	**Passive Transport**		**Active transport**
		Simple diffusion	**Facilitated diffusion**	
A	Membrane constituents responsible for transport	Proteins	Proteins	Lipids
B	Binding of the substance to be transported	Yes	Yes	No
C	Source of energy	Concentration gradient	Concentration gradient	ATP hydrolysis
D	Sensitiveness to oxidative metabolism inhibitors	Yes	No	No
E	Sensitiveness to denaturation or removal of membrane proteins	No	Yes	Yes
F	Direction of transport	In favour of the gradient of substance to be transported	In favour of the gradient of substance to be transported	Against the gradient of substance to be transported
G	Degree of specificity	Nonspecific	Specific	Specific
H	Saturation with high concentrations of the molecule to be transported	No	Yes	Yes

Q7

Most plants are monoecious species, having complete flowers that allow for self- pollination to occur. However, there are several incompatibility mechanisms present in different families to prevent inbreeding.

One of the most described systems, entitled GSI, or Gametophytic Self-incompatibility, is based on allelic composition from the mother plant (transmissive tract) and the pollen tube; if there is recognition of the self, the pollen tube stops growing and dies.

Briefly, the self-incompatibility mechanism is activated when the pollen tubes reach 1/3- 2/3 of the total distance in the style, coincident with the entry of nutrients from the transmissive tissue (or transmitting tract) to the pollen tube.

The mechanism is based on the uptake of S-RNase by the pollen tube.

Q7.1

Considering the explanation above, **indicate whether each of the following statements is true (T) or false (F).** (*4 points, 1 point each correct answer*)

A. The RNases are of maternal origin.
B. The RNases degrade pollen RNA, leading to cessation of tip growth in case of self- recognition.
C. The RNA enters the cell through the endocytosis pathway.
D. The S-RNase is degraded in case of identical alleles.

Q7.2

Considering this type of self-incompatibility, **indicate whether each of the following statements is true (T) or false (F).** (*4 points, 1 point each correct answer*)

A. The pollen haplotype S3 would be rejected in a S2S2 pistil.
B. The pollen haplotype S3 would be rejected in a S2S3 pistil.
C. The pollen tube metabolism changes from autotrophic to heterotrophic in the transmitting tract in the pistil
D. Self-incompatibility mechanisms are very important to ensure genetic variability.

Q8

Fungi are everywhere, including in the marine environments (sandy beaches, salt marshes and mangroves). Considerable efforts have been made in documenting their diversity, ecology, phylogeny and biotechnological applications. The marine fungal distribution comprises

temperate, tropical, arctic and cosmopolitan species. The majority are Ascomycota, presenting microscopic fruit bodies and spores, mostly with appendages or mucilaginous sheaths (**Figure 1**).

Marine fungi play a key role in marine ecosystems' balance and dynamic. A wide range of organic to inorganic substrates are colonized by marine fungi: wood, halophytes, seaweeds/algae, marine animals, sand, sea foam and

sediments.

Figure 1 – Microscopic photos of marine fungi: ascospores with appendages (1-2) and ascospores with sheaths (3-4).

Indicate whether each of the following statements is true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. Fungi major ecological role is the decomposition of organic matter
B. Sheaths and appendages of spores are morphological adaptations to marine life
C. Temperature and salinity are not important factors on marine fungal distribution
D. Some marine fungi produce extracellular enzymes that enable them to break down lignocellulosic materials.

Q9

In the 1850s and 1860s, the French chemist and microbiologist Louis Pasteur became the first scientist to study fermentation, when he demonstrated that this process was performed by living cells.

Fermentation processes to produce bread, wines, beers and ciders are traditionally carried out with *Saccharomyces cerevisiae* strains, the most common and commercially available yeast.

In nature, they are found on the surface of fruits and leaves. *Saccharomyces cerevisiae* is an Ascomycota fungus that reproduces asexually by budding and sexually by ascospores.

The fermentation biochemical pathway is illustrated in **Figure 2**.

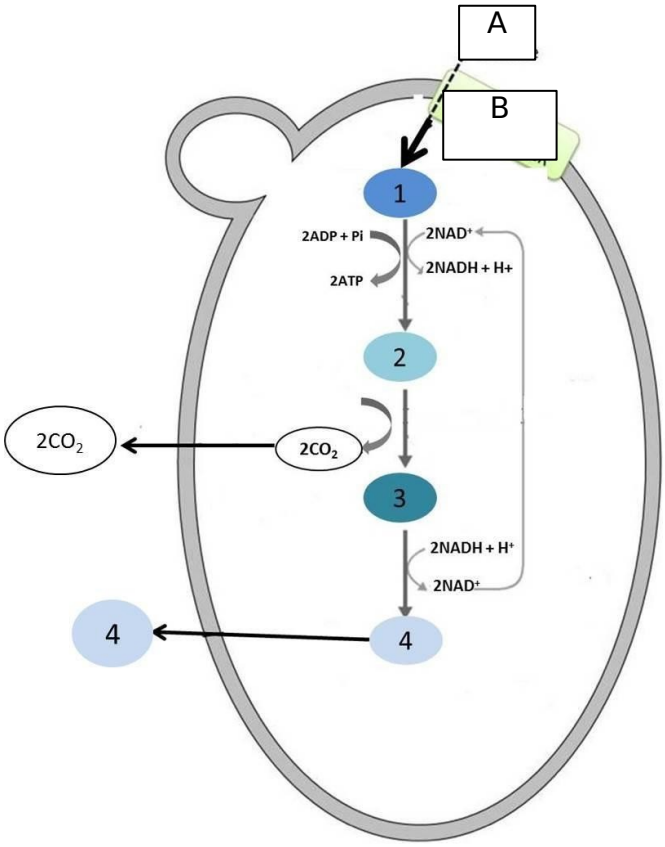


Figure 2 - Central metabolism of fermentation in yeasts. 1. Glucose. 2. Pyruvic acid. 3. Acetaldehyde. 4. Ethanol. A. Glucose; B. Hexose transporter

Indicate whether each of the following statements is true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. The oxygen presence determines pyruvate oxidation via tricarboxylic acid cycle to carbondioxide and water.

B. The main components of *S. cerevisiae* cell wall are cellulose and hemicellulose.

C. Vegetative cells multiply by budding but on nitrogen starvation may give rise to asci in which meiosis occurs to give four haploid ascospores

D. During fermentation CO_2 is removed from pyruvate and 38 ATP are formed per one molecule of glucose.

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Q10

Researchers from Harvard University show that the concentration of nutrients in the environment can set the strength of interactions between species of a microbial community. An important environmental parameter that all microbes influence and are influenced by is pH. The change in environmental pH by bacterial species was measured at high and low nutrient concentrations (**Fig. I**). Different soil bacteria were grown at low and high nutrient concentrations, then that spent media was used to re-grow each of the species in the spent media of the others (**Fig**. **II**). To determine the consequence of these environmental modifications on the coexistence of bacterial pairs, the scientists co- cultured all pairwise combinations of the eight species in batch culture with daily dilution in both low and high nutrient concentrations, then the cultures were assayed by counting the bacteria colonies (**Fig**. **III**).

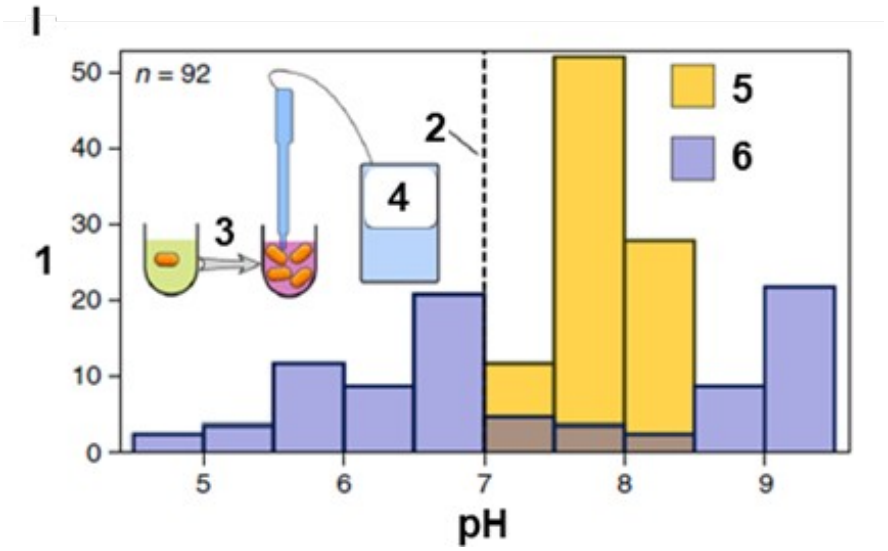


Figure I. pH – Final pH. 1. Number of strains; 2.Initial pH 7; 3.Growth; 4.Final pH; 5. Low nutrient; 6. High nutrient.

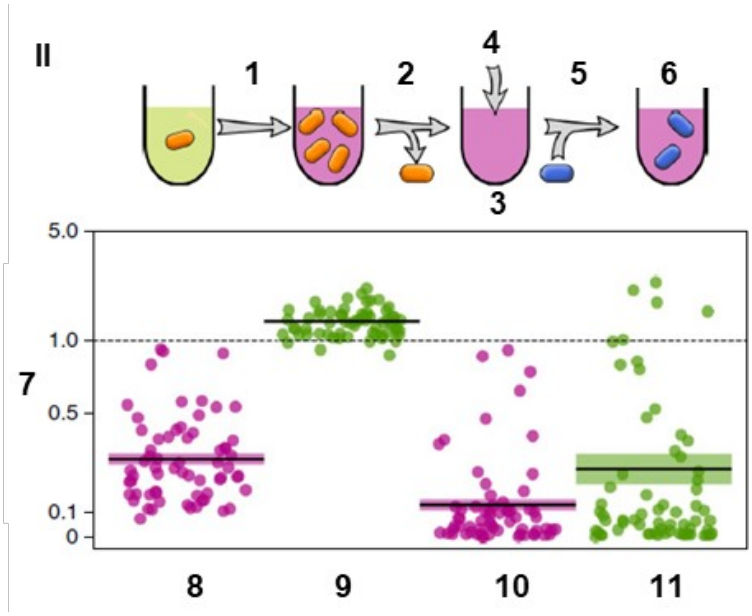


Figure II.1. Growth; 2. Remove bacteria; 3. Spent media; 4. ± fresh nutrients; 5. Add bacteria; 6. Regrowth; 7. Final OD* spent media / final OD* fresh media; 8. Low nutrient;

9. Low nutrient replenished; 10. High nutrient; 11. High nutrient replenished.

**OD = Optical Density.*

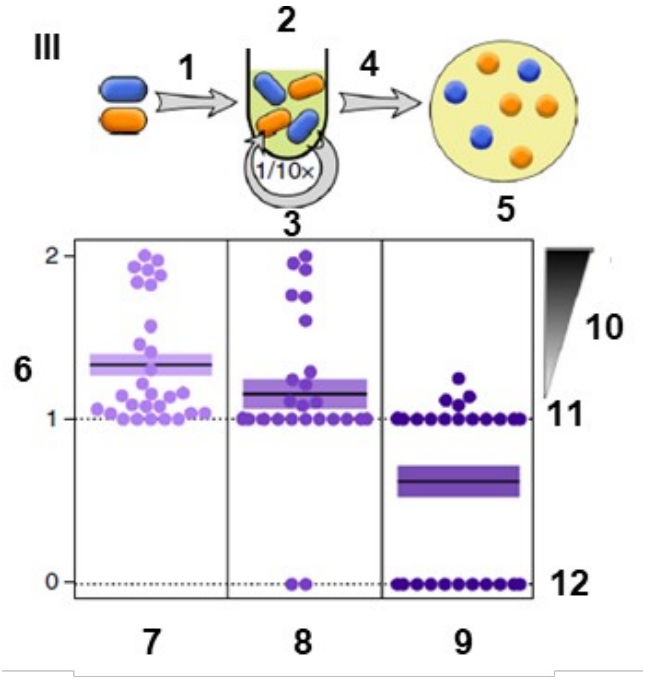


Figure III.1. Mix; 2. Cultivate; 3. Daily dilution; 4. Plate; 5. Count colonies; 6. Diversity;

7. Low nutrient 10 mM PO_4; 8. High nutrient 100 mM PO_4; 9. High nutrient 10 mM PO_4;

10. Coexistence; 11. One species wins; 12.Extinction.

After a close evaluation of the results, **indicate if each of the following statements is true (T) or false (F).** (*4 points, 1 point each correct answer*)

A. At high nutrient concentrations bacteria either increased or decreased the pH.
B. Spent media from high nutrient concentrations repressed bacterial growth completely in many cases and this was driven by resource competition.
C. An increase in nutrient concentrations led to stronger negative interactions, resulting in a loss of coexistence
D. Higher buffer concentrations prevented the loss of coexistence at high nutrient concentrations, showing that pH is a major driver of the species interactions in this system.

Q11

~~Bacteria are everywhere and if the conditions are suitable they multiply. If growth is not possible, nongrowing states can be adopted. Two of those states are spores and viable but nonculturable (VBNC) cells.~~

~~**Indicate whether each of the following statements is true (T) or false (F).** (*4 points, 1 point each correct answer*)~~

~~A. Bacterial spores and VBNC cells allow bacteria to cope with unsuitable growth conditions and resume growth later.~~
~~B. A viable bacterial cell is always culturable~~.
~~C. Bacterial spores and VBNC cells represent viable cells, and they can grow in most media commonly used in microbiology laboratories~~
~~D. The recognition of dormant cells led scientists to hypothesize that sometimes those cells are responsible for the same disease, later in life, in a formerly healed patient.~~

Q12

Prebiotics function as dietary fibers that are not digestible in the upper gastrointestinal tract and promote the growth of intestinal bifidobacteria. Inulin is a prebiotic that originates from plants. Like starch, it is a plant storage product, but unlike starch it cannot be stained with Lugol's. In the pharmaceutical industry, it is extracted from the root of chicory (*Cichorium intybus*). Inulin's low caloric content permits it to replace sucrose.

Select from **Table 1**, the number corresponding to the appropriate word, to

complete the blank spaces from A to J in the text. (**More than one option may be correct**)

(*10 points, 1 point each correct answer*)

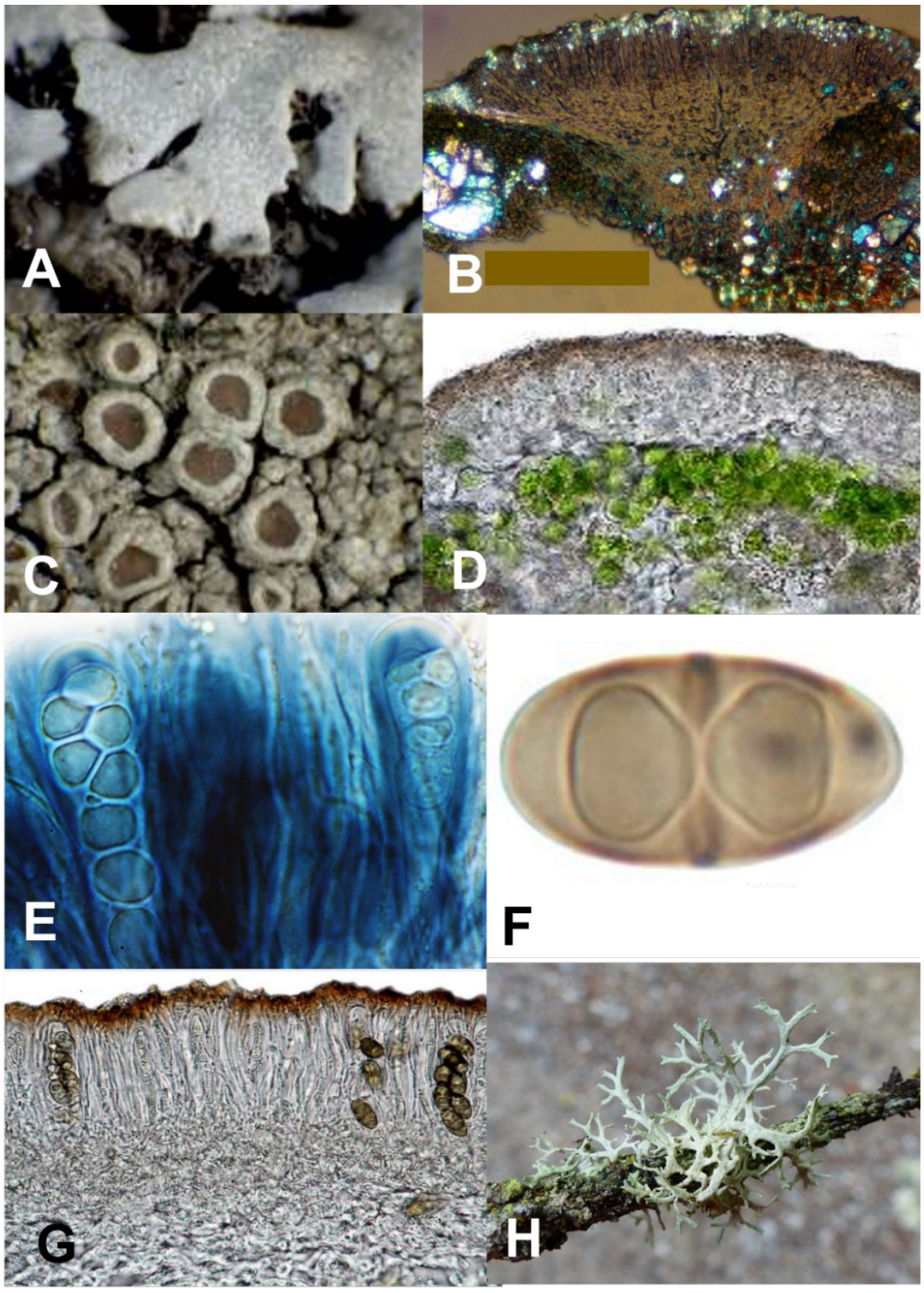
Table 1			
1. Inulin	6. Vacuole	11. Endoplasmic reticulum	16. Acidity and hydrolysis
2. Lipids	7. *Cichorium intybus*	12. Starch	17. Polysaccharides
3. Cell wall	8. Sweetener	13. Fibers	18. Digestible
4. Amyloplasts	9. Plastids	14. Pharmaceutical	19. Cosmetics
5. Carbohydrates	10. Proteins	15. Aromatherapy	20. *Daucus carota*

Some examples of plants that contain large amounts of inulin are Jerusalem artichoke (*Helianthus tuberosus*), chicory root [**A**…], garlic (*Allium sativum*), asparagus root (*Asparagus officinalis*), among others. Inulin has been used as a low calorie [**B**…], to form gels, increase viscosity, improve aroma and taste properties and as a non [**C**…] [**D**…]. As a [**E**…] inulin is used as a diagnostic tool to measure kidney function. Cellulose and starch are the most common [**F**…] of plants, but [**G**…], xylans, and hemicelluloses are also examples of this group. However, inulin does not accumulate in [**H**…], like cellulose, or in plastids like [**I**…]. Under physiological conditions, inulin is in solution. By artificial dehydration, numerous crystals are formed that aggregate, giving rise to fan shaped structures, which remain inside the [**J**…], surrounded by the tonoplast.

II. Plant Anatomy and Physiology

Q13

A description of three lichen species is provided. Associate each figure (**A-H**) to the corresponding species description (**A, B and C**) based on anatomical structures and morphological characters. (*8 points, 1 point each correct answer*)



DESCRIPTION:

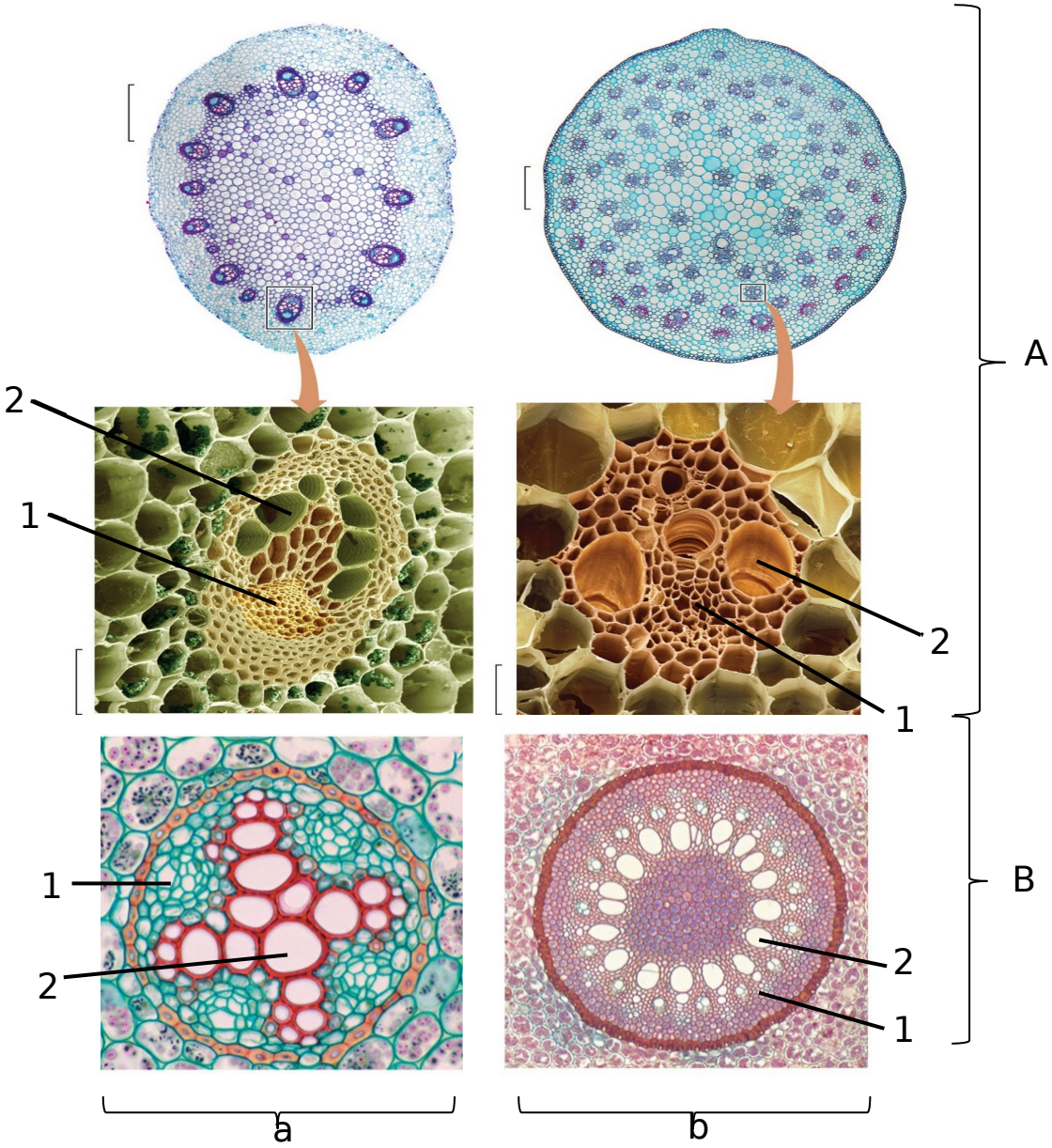
Species A - Thallus crustose, smooth to slightly verrucose, white to pale grey. Apothecia with a pale brown disc and a thick, smooth to usually crenulate margin. Margin of the apothecia (in section) with abundant, very large crystals. Asci with 8 spores, elongate Ascospores 1-celled, translucent, broadly ellipsoid.

Species B - Thallus foliose to fruticose, erect and tufted to pendent, to 10 cm long (usually less), not rigid; branches flattened, strap-shaped to almost linear, green to yellowish green. Apothecia. Photobiont: chlorococcoid (green algae).

Species C - Thallus foliose, narrow-lobed, closely adpressed, forming orbicular rosettes to 5 cm diameter. Lobes up to 2 mm broad, flat to slightly convex, whitish grey, distinctly white-maculate. Apothecia common, to 3 mm across, with a white-pruinose disc and a smooth margin. Asci 8-spored. Ascospores: 1-septate, brown, ellipsoid.

Q14

The figure shows the vascular tissue of young stems and roots (capital letters) of two groups of plants, dicotyledonous (eudicots) and monocotyledonous (monocots) (lowercases).



Indicate whether each of the following statements is true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. The vascular systems of stems differ from those of roots. In a root, the vascular tissue lies deep in the interior, with the xylem at or near the centre.
B. In monocots, the vascular bundles in the stem are arranged in concentric circles while in eudicots they are scattered.
C. Each vascular bundle contains both xylem and phloem. The xylem in vascular bundles in stems and roots is indicated with the number (1) and the phloem with the number (2).
D. In addition to the vascular tissues, the stem contains other important storage and supportive tissues. In eudicots the pith lies inside the ring of vascular bundles and also extends between them, to the outside, the cortex, which may contain supportive collenchyma.

Q15

Ammophila arenaria, also known as marram grass or European beachgrass is a plant species that grows in beach sand dunes. The work “The adaptive power of *Ammophila arenaria*: biomimetic study, systematic observation, parametric model and experimental tests” recently published in Polymers (Andrade *et al.* 2021) explores the potential of the
A. arenaria reversible leaf opening and closure mechanism that acts in response to stress to create responsive facade solutions in architecture (biomimicry).

Mechanical/technical models can be made to mimic the *A. arenaria* leaf behaviour.

Figure 1.a) Shows the leaf characteristics responsible for the leaf movements in response to stress. The figure represents leaf transverse section, showing the abaxial surface (**ab**) and the adaxial surface (**ad**) in conditions of water availability (**W**) and water stress (**WS**) and the motor cells in the adaxial surface (**TBC** – turgid bulliform cells and **FBC** – flaccid bulliform cells).

Figure 1.b) Shows the thermobimetal response to changes in temperature. A – active layer, B – passive layer.

Figure 1.c) Shows the prototype material based on the combined use of *A. arenaria* and bio-inspired thermometal materials at two distinct

temperatures.

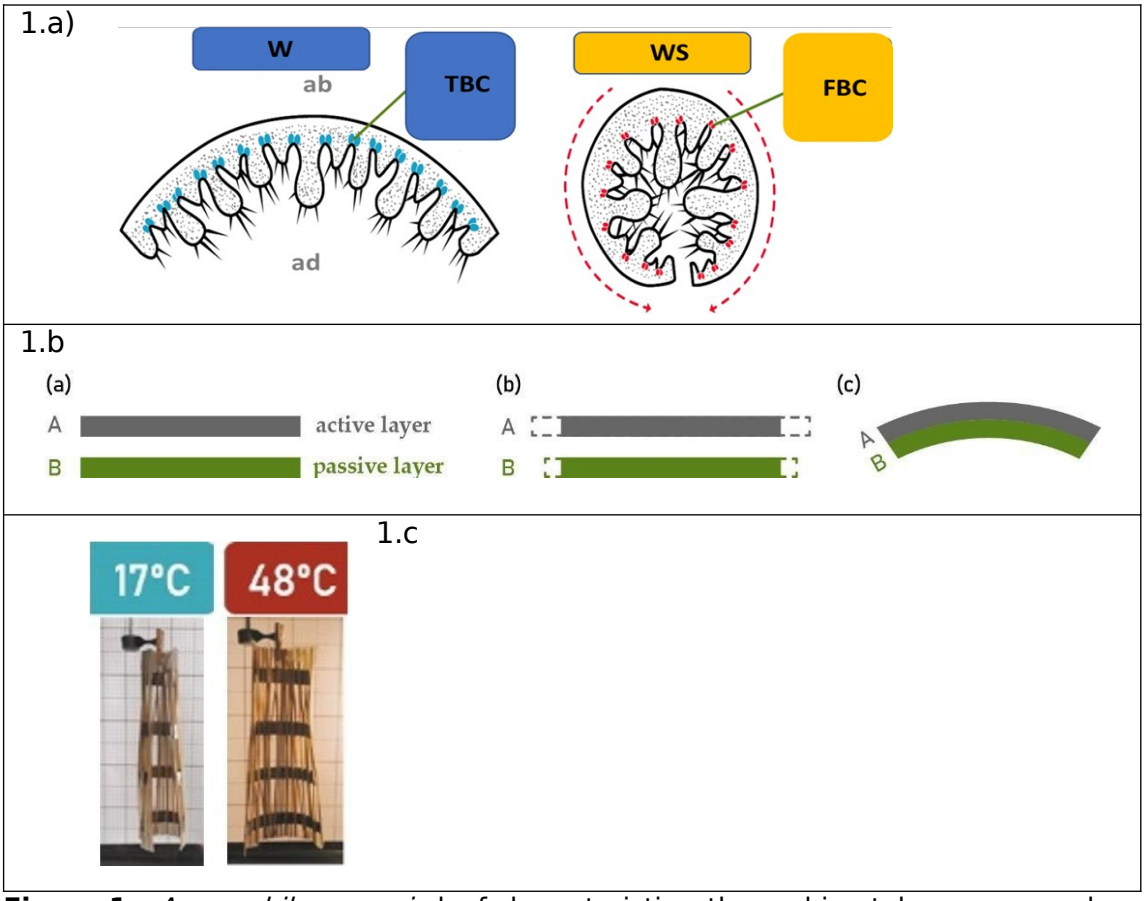


Figure 1 - *Ammophila arenaria* leaf characteristics, thermobimetal response and thermometal materials. 1.b Active layer = (insert your translation), Passive layer =. (insert your translation)

Indicate whether each of the following statements is true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. Leaf turgid bulliform cells are crucial in the leaf opening and closing in response to water stress.
B. Comparing the functional surfaces of leaf and thermobimetal materials we can say that the leaf adaxial face corresponds to the active layer of the thermobimetal material.
C. The model/prototype build with both materials showed an increased area in face of an increased temperature and therefore have potential to be used to shade building facades
D. The results show that the use of thermobimetal materials to cover building facades increases facade temperatures from 17 to 48 °C.

Q16

Serendipita indica is a non-obligate endophytic fungus, known generally a plant growth and defence promoter with high potential to be used in agriculture. The work by Dias *et al.* (2020) published in Frontiers in Microbiology explores how carbon (C) availability in the growth medium during the free-living stage of *S. indica* can influence its phenotype. Further, this study shows that the growth conditions leave a legacy to the symbiosis stage and regulate *S. indica*'s potential to promote plant growth.

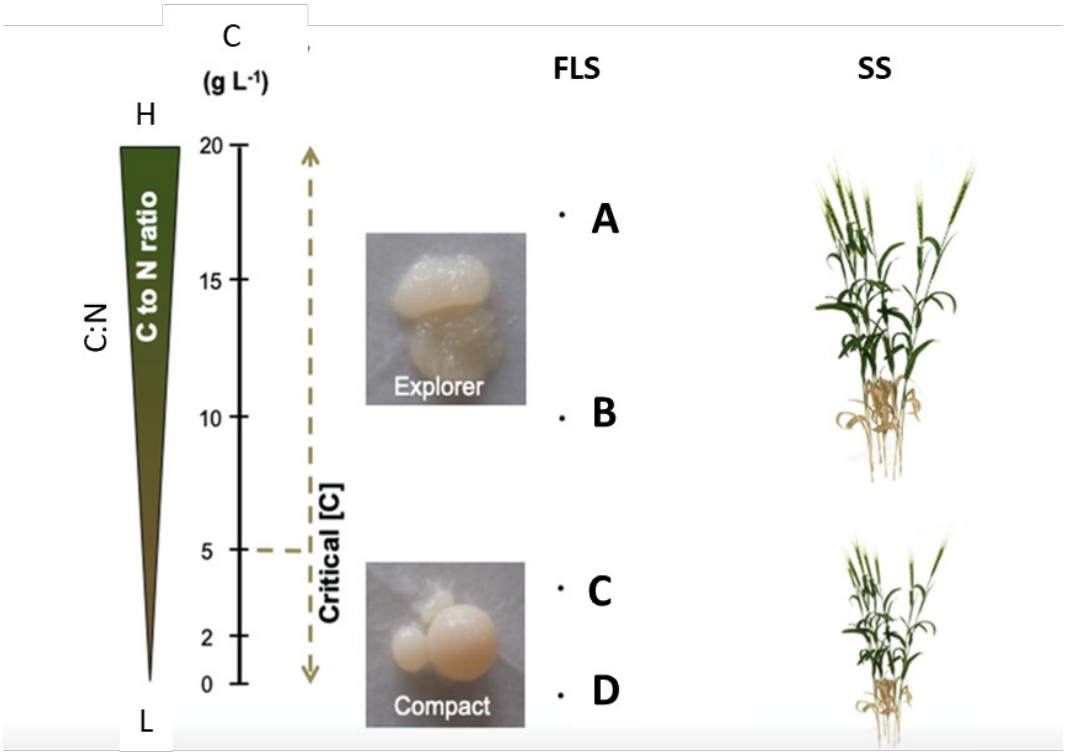


Figure 2 - Carbon (C) availability in the growth medium triggers nutrient imbalances which regulate *Serendipita indica*'s phenotype during the free-living stages and symbiosis stages (when later colonising the plant host). FLS: Free-living stage. SS: Symbiosis stage. L: Low. H: High, **A**: Glucose/sucrose is the main C source; casein and yeast extract are also the N sources, **B**: N and P scavenging, **C**: Peptone is the main C and N source, **D**: C scavenging.

Indicate whether each of the following statements is true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. *S. indica*'s compact phenotype develops when the fungus is submitted to carbon deprivation.
B. *S. indica*'s explorer phenotype displays higher capacity to decompose carbon containing molecules.

C. Under high C/N ratio in the growth medium, *S. indica* obtains the distinct nutrients from different sources.
D. The N availability during the free-living stage is likely to leave a legacy to the symbiosis stage, regulating *S. indica*'s potential to promote plant growth.

Q17

The work by Ramos *et al.* (2020) published in Planta explores how inoculation with the endophytic plant-growth promoting bacterium (PGPB) *Herbaspirillum seropedicae* can be a strategy for promoting growth, nutrient uptake (Table 1) and photosynthetic efficiency (**Figure 3**) in rice (*Oryza sativa*).

Table 1 – Effect of *H. seropedicae* inoculation on rice shoot mineral contents. * Marks significant effects at $p \leq 0.05$. Values are means ± SE (n = 5). **NI** – Non-inoculated,

I - inoculated, **IE** - Inoculation effect, **Ma** – Macronutrients, **Mi** - Micronutrients

	NI	**I**	**IE**
Ma (mg shoot^{-1})			
*	15 ± 1	33 ± 1	120%
*	1 ± 0	6 ± 1	+350%
*	14 ± 1	77 ± 1	+430%
Ca *	4 ± 1	11 ± 1	+150%
Mg *	3 ± 0	6 ± 1	+110%
*	2 ± 0	6 ± 1	+300%
Mi (µg shoot^{-1})			
Fe *	323 ± 12	576 ± 19	+80%
*	15 ± 1	50 ± 2	+230%
Cu *	10 ± 1	39 ± 0	+280%
Mn *	682 ± 28	465 ± 17	30%
Mo *	0 ± 0	2 ± 0	+1020%
Ni *	11 ± 0	50 ± 1	+340%
Zn *	95 ± 13	3385 ± 193	3460%

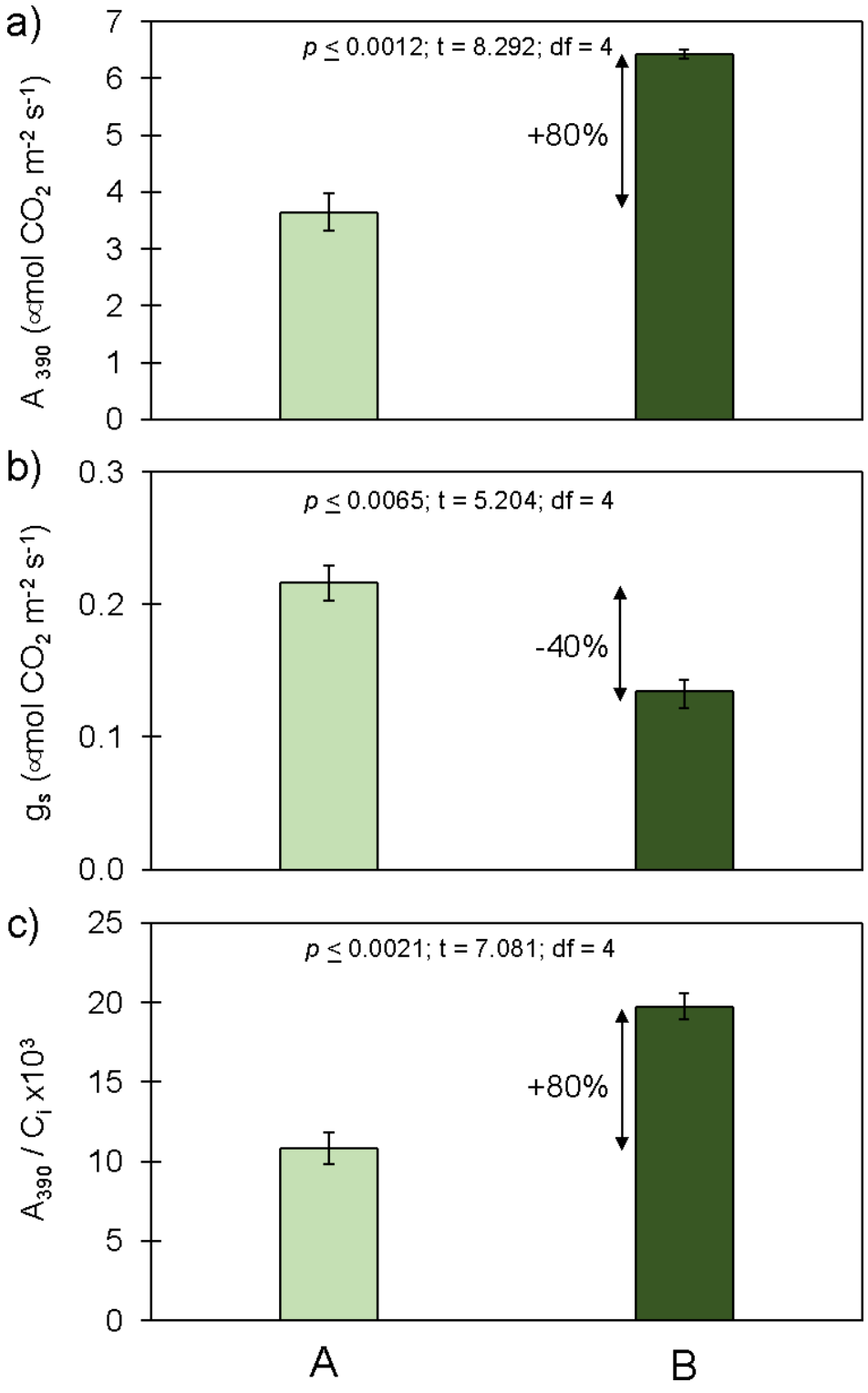


Figure 3 – Effect of *H. seropedicae* inoculation on rice photosynthetic activity. Photosynthetic activity was evaluated by determining (a) net carbon assimilation at ambient CO_2 concentrations (A_{390}), (b) stomatal conductance (g_s), and (c) carboxylation efficiency (A_{390}/Ci x 103). Bars are means ± SE (n = 5). A- Non-inoculated; B- Inoculated.

Indicate whether each of the following statements is true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. The effect of *H. seropedicae* inoculation on rice nutrient uptake was similar for all nutrients.

B. *H. seropedicae* inoculation resulted in higher stomatal resistance to CO_2 diffusion.

C. For the same internal CO_2 concentration, rice plants inoculated with *H. seropedicae* assimilated less carbon.

D. Rice plants inoculated with *H. seropedicae* had a higher nutritional value for human diet.

Q18

Global wheat yield is affected by climatic variation, including water deficit and heatwaves. The work "Photoprotection and optimization of sucrose usage contribute to faster recovery of photosynthesis after water deficit at high temperatures in wheat" (Correia *et al.* 2020), presents results obtained with two wheat genotypes (Paragon and Sokoll) under water deficit and elevated temperature, in isolation or combination, and part of that results are used here. Table 1 shows plant water status estimated by leaf relative water content (LRWC) and leaf water potential (LWP), and Figure 4 the rate of net photosynthetic CO_2 assimilation, stomatal conductance and electron transport in the photochemical reactions of photosynthesis.

Table 1 Leaf water status, and canopy temperature of Paragon and Sokoll wheat plants exposed to a combination of heat stress and water deficit and recovery from heat stress conditions.

		Genotype	LRWC (% H_2O)	LWP (MPa)	Tcanopy (°C)
25°C	WW	Paragon	90.11 ± 8.82 c	−0.50 ± 0.08 c	26.87 ± 0.65 a
		Sokoll	90.20 ± 1.73 c	−0.81 ± 0.12 bc	26.33 ± 0.19 a
	WD	Paragon	68.24 ± 12.45 b	−1.16 ± 0.16 ab	28.79 ± 0.62 b
		Sokoll	31.89 ± 8.87 a	−1.39 ± 0.10 a	27.89 ± 1.10 b
38°C	WW	Paragon	78.60 ± 8.47 bc	−0.82 ± 0.06 bc	35.04 ± 0.98 c
		Sokoll	80.38 ± 4.74 bc	−0.77 ± 0.09 bc	33.37 ± 0.40 d
	WD	Paragon	39.60 ± 17.71 a	−1.30 ± 0.59 a	36.95 ± 0.74 e
		Sokoll	43.06 ± 26.64 a	−1.55 ± 0.58 a	37.52 ± 0.47 e
Recovery	RWW 38°C	Paragon	86.46 ± 1.36 c	−0.76 ± 0.03 bc	25.71 ± 0.3 a
		Sokoll	94.91 ± 4.82 cd	−0.74 ± 0.05 bc	25.58 ± 0.4 a
	RWD 38°C	Paragon	90.83 ± 3.42 c	−0.72 ± 0.1 bc	26.33 ± 0.44 a
		Sokoll	78.31 ± 21.18 bc	−0.98 ± 0.16 ab	26.43 ± 0.21 a

***Note*:** Plants were grown for 3 weeks, then exposed to heat stress (38ºC vs. control, 25ºC), water deficit (WD vs. well-watered WW) and re-watered at control temperature (25ºC) after heat stress conditions (RWW38ºC and RWD38ºC). Values are means ± SD (n = 5 biological replicates). Different letters denote statistically significant differences between treatments (Duncan analysis, $p < 0.05$). Abbreviations: LRWC, leaf relative water content; LWP, leaf water potential; Tcanopy, canopy temperature.

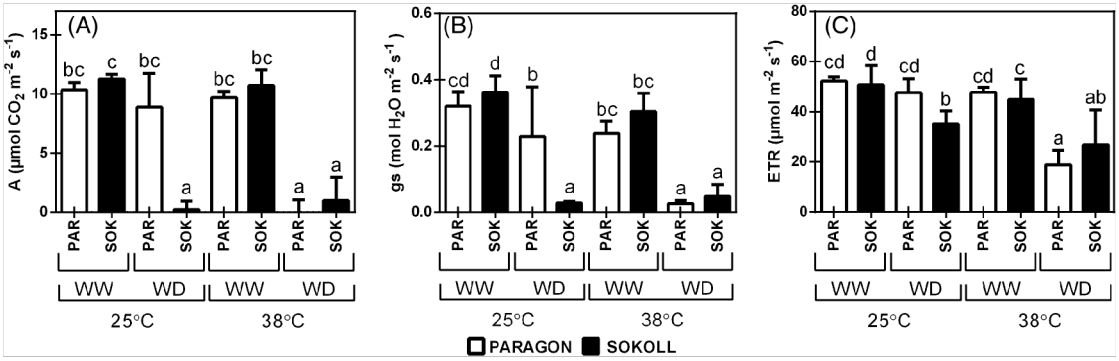


Figure 4 - Steady-state photosynthesis of Paragon (PAR) and Sokoll (SOK) wheat plants exposed to a combination of heat stress and water deficit. (A) Net CO_2 assimilation, (B) stomatal conductance (gs) and (C) electron transport rate (ETR) were measured at growth light and ambient CO_2 in fully expanded leaves of wheat 3-week-old plants under well-watered (WW) and water deficit (WD) conditions and exposed to control (25ºC) and heat stress conditions (38ºC). Values are means ± SD (n = 5 biological replicates). Different letters denote statistically significant differences between treatments (Duncan analysis, $p < 0.05$)

Based on the text, **Table 1** and **Figure 4**, decide if the following sentences are **true (T) or false (F).** (*4 points, 1 point each correct answer*)

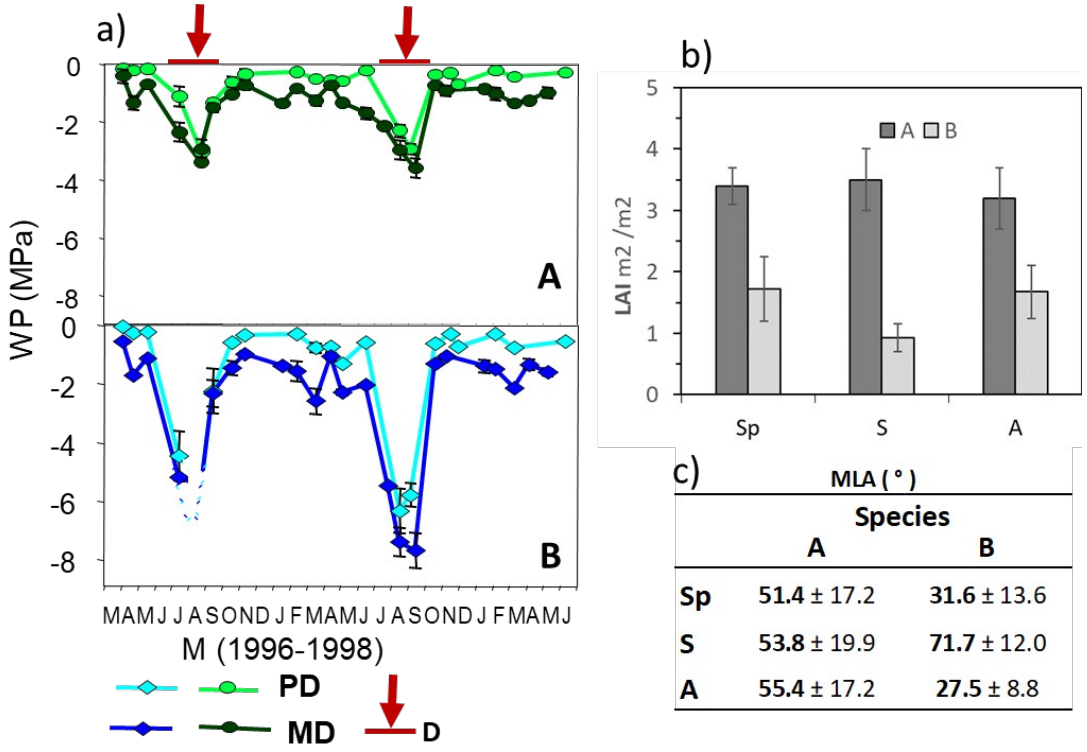
A. Plant water deficit (WD) conditions increased more the driving force for water movement through the plant in wheat genotype Paragon than in genotype Sokoll.
B. Under plant well-watered (WW) conditions, wheat genotype Paragon will avoid cellular heat stress better than genotype Sokoll
C. Under plant water deficit (WD) conditions, without heat stress, net photosynthesis of genotype Paragon is maintained, as opposed to Sokoll, and the higher capacity to conserve cellular hydration observed in genotype Paragon can contribute to this.

D. With the increasing occurrence of heatwaves and drought in Mediterranean region a relevant decrease is expected in vegetative growth of wheat genotypes Paragon

and Sokoll, caused by limitations in net photosynthetic rate at photochemical level and CO_2 assimilation. .

Q19

The plant community of the Mediterranean maquis comprises mainly two functional groups: the evergreen sclerophylls and the drought semi-deciduous species, which differ in their physiological and structural adaptations to environmental stress factors. **Figure 5** shows the seasonal variation of leaf water potential (WP) at pre-dawn and midday (a), Leaf Area Index (LAI) (b) and leaf angles relative to the horizontal (MLA) (c) in different seasons for evergreen sclerophylls and drought semi-deciduous species.



c)

MLA (°)

Species	A	B
Sp	51.4 ± 17.2	31.6 ± 13.6
S	53.8 ± 19.9	71.7 ± 12.0
A	55.4 ± 17.2	27.5 ± 8.8

Figure 5. A, B – Two different species A and B; **a) WP** - Leaf water potential, **PD** - Pre- dawn water potential, **MD** - Midday water potential, **D** - Drought period in summer **b) LAI** - Leaf area index (m2 m-2), **c) MLA** - Mean leaf angle (° from the horizon). **Sp** - Spring, **S** - summer, **A** - autumn.

Indicate whether each of the following statements is true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. Species A is considered a drought semi-deciduous species since it partially avoid stress through higher water potentials
B. In species B the pronounced leaf shedding during summer, which may reach 50%, involves some advantages for water economy, by reducing the transpiring surface during the period of maximum water stress
C. Predawn water potentials decline below -6 MPa in species B due to their deep and well-developed root system which can reach water at depth.
D. Species B exhibits pronounced structural changes in leaf architectures, reducing the interception of excessive radiation during the summer drought period, avoiding photoinhibition.

Q20

Lichen species differ in their tolerance to environmental nitrogen availability, with some species tolerating high atmospheric concentrations of ammonia (NH_3) and others disappearing at low ammonia concentrations.

Chlorophyll *a* fluorescence is a parameter commonly used as a vitality index of photosynthetic organisms, providing information about the physiological performance of the organism. Approximately, this index varies between 0.8 (optimal value = healthy conditions) and 0 (high stress conditions = complete inhibition of the system). It is commonly used to investigate lichens' response to nitrogen excess.

Consider **Species1**, a nitrogen-tolerant lichen species, and **Species2**, a nitrogen- sensitive one.

Figure 6 shows the frequency of the two species along a gradient of ammonia; **Figure 7** shows the physiological performance of the two species indicated by the vitality index.

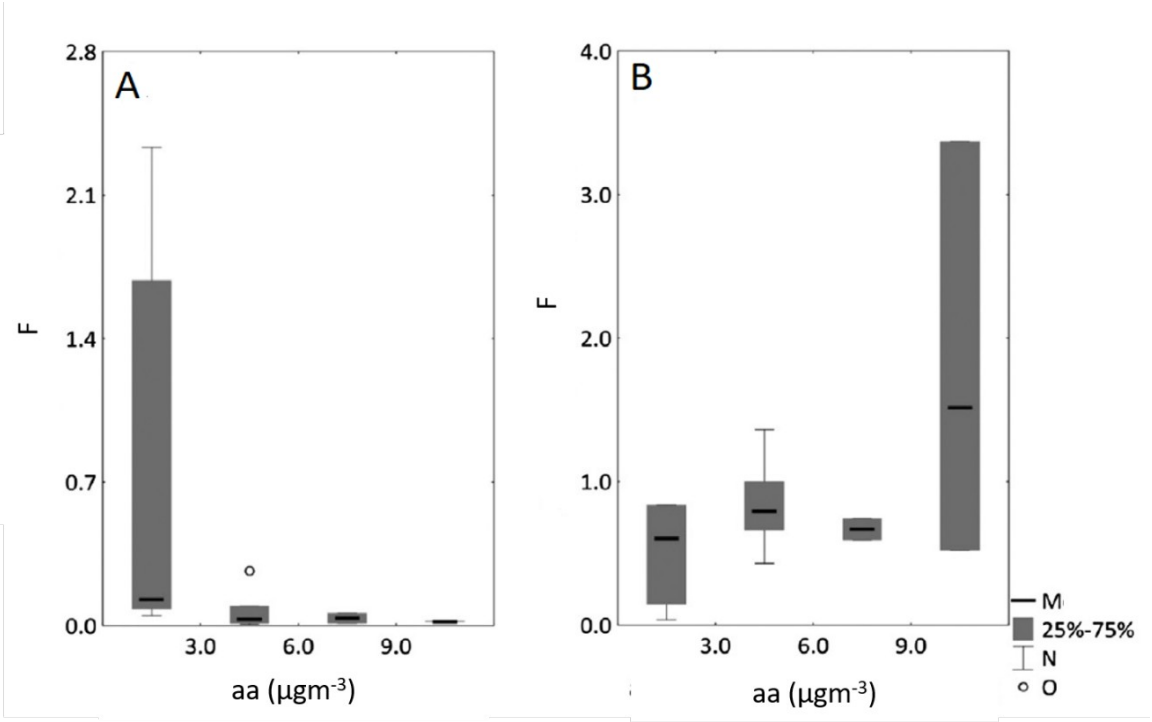


Figure 6. F = Frequency; aa = atmospheric ammonia; M = median; N = Non-Outlier Range; O = Outliers

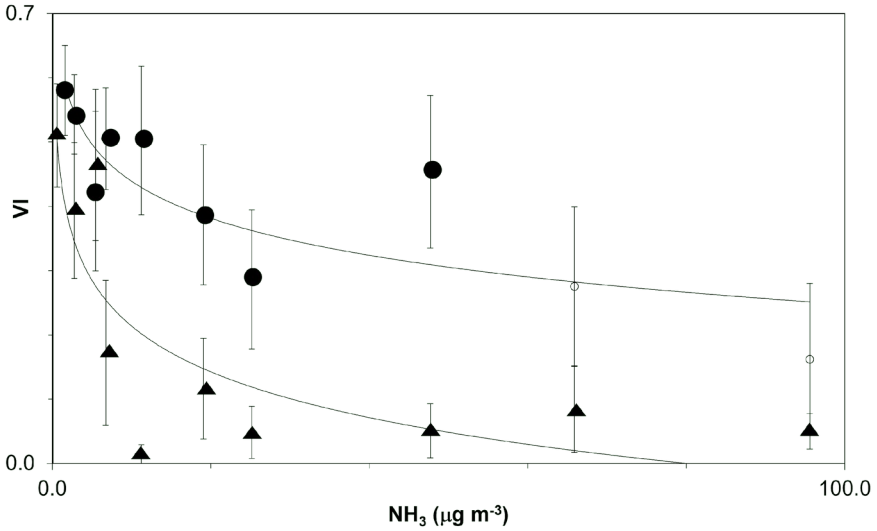


Figure 7. VI = Vitality Index

Lichens have several physiological mechanisms of nitrogen tolerance. One of them is to store excess nitrogen in non-toxic forms, like chitin, inside their tissue. Chitin can incorporate nitrogen in excess since nitrogen represents around 6.3% of chitin’s mass.

Table 1 shows two values obtained for the concentration of chitin and nitrogen in the tissue of the two species.

	Chitin (mg g^{-1})	Total N (mg g^{-1})
Value 1	3.40 ± 1.42	12.34
Value 2	20.74 ± 3	23.37

Q20.1

Select the correct answer scheme to associate each parameter to **Species1 or Species2**.

(*5 points, 1 point each correct answer*)

	Species 1	Species 2
Graph A, fig. 6		
Graph B, fig. 6		
Circle, fig. 7		
Value 1, tab. 1		
Value 2, tab. 1		

Q20.2

Indicate whether each of the following statements is true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. Increased NH_3 promotes an enhanced physiological performance in one of the species
B. Intermediate values of ammonia concentration increase lichen frequency.
C. Nitrogen pollution affects competition dynamics among lichen species.

D. The difference in the nitrogen content in the two species is exclusively due to the difference in chitin content

Q21

Figure 8 shows that some plants require a prolonged cold period (vernalization) as experienced during winter (W), before they will flower (**Fig. A**). The flowering time is controlled by a "well characterized" epigenetic mechanism that targets FLOWERING LOCUS C (FLC). This FLC encodes a MADS box transcription factor that binds and represses the FT gene. Thus, the mutant *flc* is characterized by a precocious flowering in autumn (A) (**Fig. B**). The molecular mechanisms by which the FLC control the flowering are illustrated in **Fig. C**.

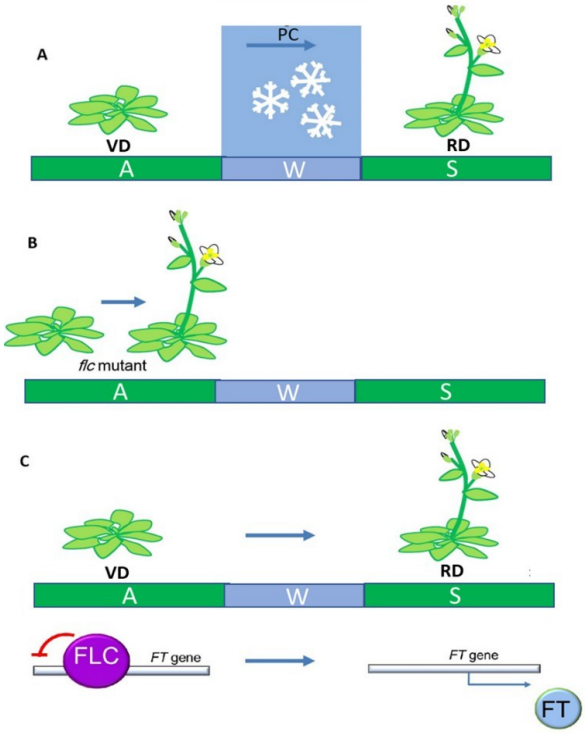


Figure 8. **A** - Autumn, **W** – Winter, **S** –Spring, **VD** – Vegetative development, **RD** – Reproductive development, **PC** – Prolonged cold.

Indicate if each of the following statements is true (T) or false (F).
(*5 points, 1 point each correct answer*)

A. FLC is an inhibitor of flowering
B. Transcription of FT gene is activated by FLC binding.
C. FT is mainly expressed in vegetative plant tissues in autumn.

D. The transcriptional activity of FLC is insluenced by a cold-sensitive epigenetic process
E. Cold induces the silencing of FLC, allowing FT to be expressed and induce flowering.

III. Animal Anatomy and Physiology

Q22

Phylum Nematoda (Roundworms) has approximately 12 000 known species, although estimates point out the existence of 500 000. They live in all habitats, from aquatic to terrestrial, from tropics to polar regions, from mountain tops to the depths of the sea. One of the most known Nematoda is *Ascaris lumbricoides*, a human parasite causing discomfort, disease and in severe cases death. A female *Ascaris* may lay 200 000 eggs a day. Females' reproductive system consists of a paired structure with two very long tubes coiled inside the organism, that can be differentiated in three regions: ovary, oviduct, and uterus with increasing diameter (**Figure 1**). In cross-section, these regions present different aspects (**Figure 2**).

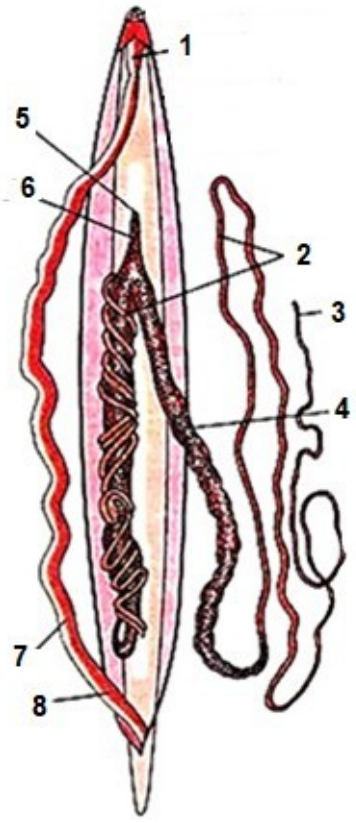


Figure 1. Structure of a Nematoda.

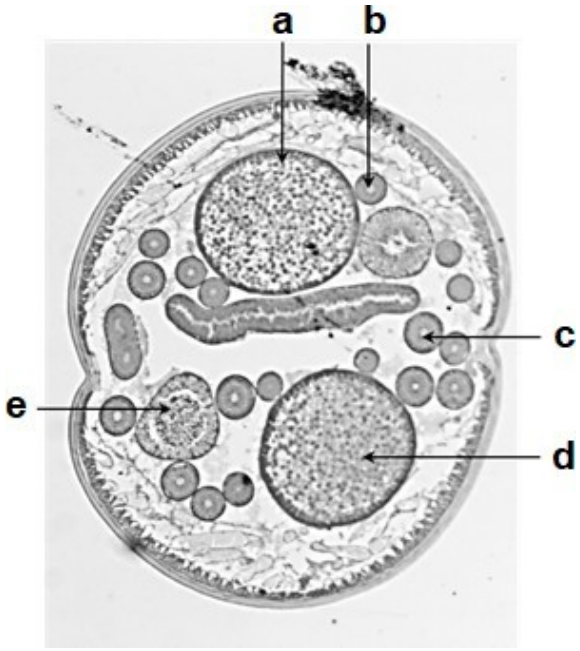


Figure 2. Cross section of *Ascaris lumbricoides*.

1. Pharynx, 2. Oviduct, 3. Ovary, 4. Uterus,
5. Vulva, 6.Vagina, 7. Intestine, 8. Rectum

Indicate whether each of the following statements is true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. The uterus is full of eggs because it is where they are collected before spawning.
B. The letters b and c correspond to the ovary, e to the oviduct, a and d to the uterus.
C. The presence of several sections of the same region of the reproductive system indicates that in this specific individual the reproductive system is haggard.
D. The letters b and c correspond to the uterus, e to the oviduct, a and d to the ovary

Q23

Circulatory systems of two Fishes (**P and Q**) are shown.

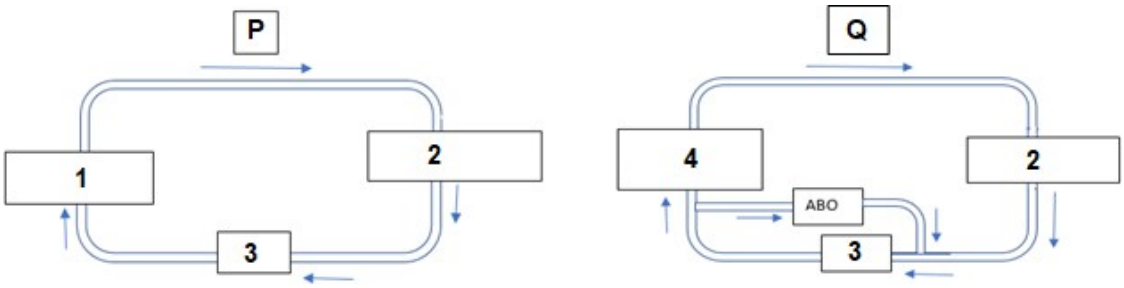


Figure 1. 1. Gills; 2. Systemic Tissues; 3. Heart; 4. Partially functional gills; ABO: Air Breathing Organ

Q23.1

Observe them carefully and mark the following statements as **true (T) or false (F).**

(*4 points, 1 point each correct answer*)

A. Oxygen saturation levels of blood reaching heart in P is higher than that in Q.
B. Under highly anoxic water body conditions, fish Q will have survival advantage over P.
C. Delivery of oxygenated blood to tissues is less in P as compared to Q.

D. Emptying of oxygenated blood from ABO to systemic arteries would improve the delivery of oxygen to body parts.

Q23.2

The heart tissue or myocardium of fishes such as P and Q can be of two types. In many bony fishes, it is entirely spongy and absorbs oxygen from the blood flowing through heart during circulation. In many other fishes, myocardium is compact (non-spongy) and oxygen is supplied to heart tissue via coronary artery. (Refer to the figure 1.)

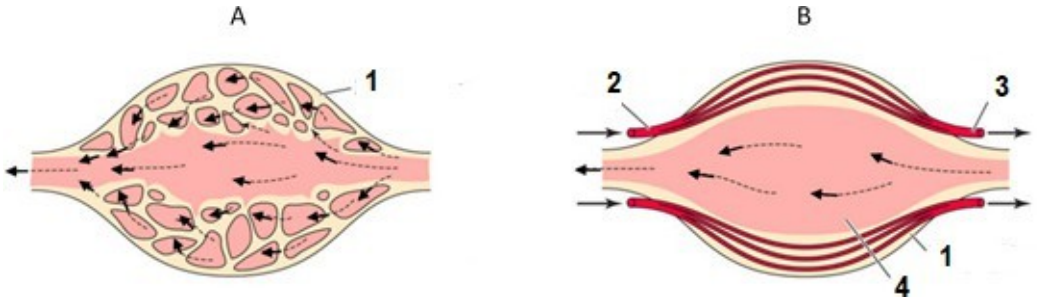


Figure 1. A. Spongy myocardium; B. Compact myocardium; 1. Myocardium; 2. Coronary artery;
3. Coronary vein; 4. Ventricular lumen. (*Reference: Animal Physiology by Hill, Wyse and Anderson.(3rd Edition)).*

Mark the statements as true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. Exercise performance or migratory abilities of fish with spongy myocardium will be limited as compared to a fish with compact myocardium.
B. For an active fish having spongy myocardium, circulatory system as found in fish Q is more advantageous than the one found in fish P.
C. Circulatory system P is more advantageous for a fish with compact myocardium tissue over a fish with spongy myocardium.
D. For a fish with compact myocardium, the extent of delivery to the tissues will be the same irrespective of the type of circulatory system.

Q24

When simultaneous deletion of two genes leads to cell death, deleting each gene individually does not. This is known as synthetic lethality. Synthetic lethal compounds have been screened in order to identify new drugs for cancer treatment, namely for breast cancer, where targeting different DNA repair pathways is a promising strategy for BRCA1/2 mutated tumors.

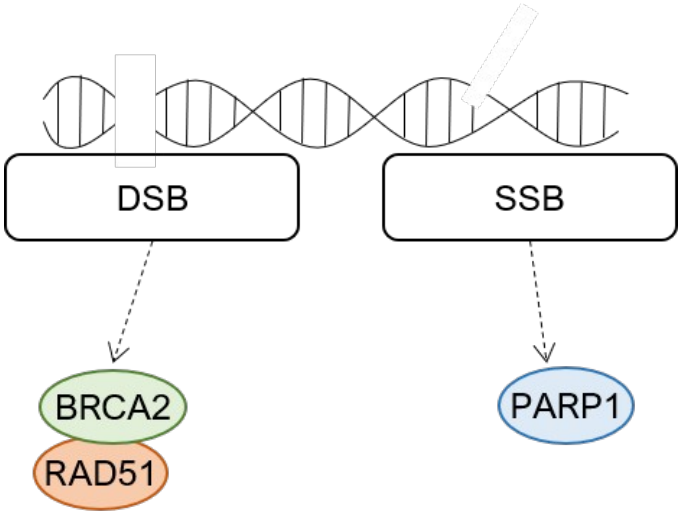


Figure 1. DNA repair. **DSB** – double strand break; **SSB** – single strand break.

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. Synthetic lethality is not a promising strategy since treatment with the identified drug may cause death of both normal and tumor cells.
B. Using an inhibitor against BRCA2 is not a successful strategy to target BRCA2- deficient tumors.
C. Inhibiting RAD51 is a possible therapeutic approach to target BRCA2 deficient tumors.
D. The use of PARP inhibitors is a synthetic lethal approach to target BRCA2 deficient tumors.

Q25

Iron is an essential nutrient for the vast majority of organisms, participating in several different reactions and acting as a co-factor of many proteins. However, when not properly regulated and stored, free iron can also be detrimental because...

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. It alters redox-state mitochondrial proteins, promoting their reduction.
B. It promotes iron intake from food.
C. It leads to the oxidation of different macromolecules, such as DNA, proteins and lipids.
D. It promotes tissue healing and regeneration.

Q26

The transcription factor p53 is a protein that regulates a variety of pathways including cell proliferation, DNA repair, cell death and metabolism. In an experiment, hepatocytes isolated from a healthy donor were cultured *in vitro* and the gene encoding p53 (*TP53*) was deleted. The impact on glycolysis and gluconeogenesis was assessed by Real Time quantitative PCR (RT-qPCR), comparing the wild type cell line (wt) and the p53 deleted cell line (ko).

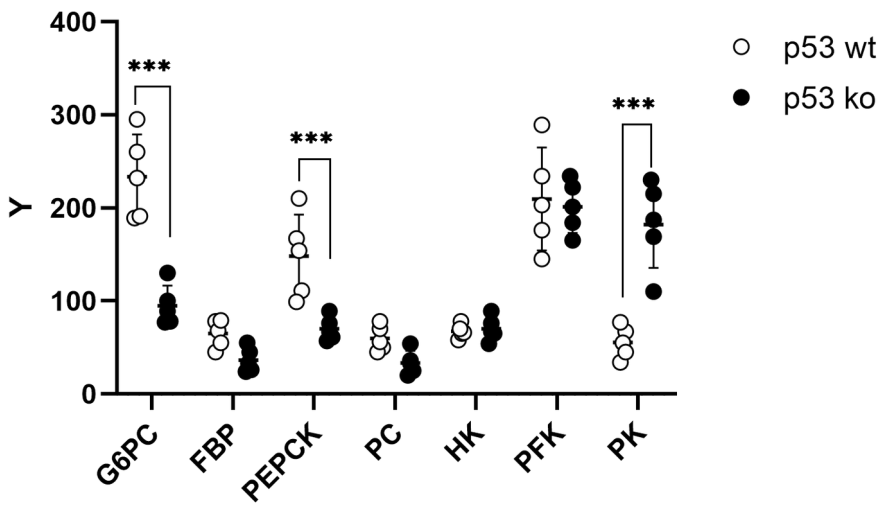


Figure 1 mRNA expression of different genes involved in glucose metabolism. Y – mRNA expression; X – Housekeeping genes G6PC – glucose-6-phosphatase; FBP – Fructose - fructose-1,6-bisphosphatase; PEPCK – PEP carboxylkinase; PC – pyruvate carboxylase; HK – hexokinase; PFK – phosphofructokinase; PK - pyruvate kinase.

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. Analysis of the graph suggests that p53 promotes glycolysis.
B. Glucose-6-phosphatase expression is regulated by p53.
C. In the absence of p53 gluconeogenesis is downregulated.
D. There is no effect of *TP53* deletion on either glycolysis or gluconeogenesis.

Q27

Under normal feeding conditions, our glucose supply is obtained from food intake. After food has been absorbed and subsequently stored, our body initiates a starvation period, initially referred as postabsorptive state. If food deprivation is extended, we enter a prolonged fasting period that can ultimately lead to death. At each stage, glucose is obtained via different strategies that are sequentially put in place.

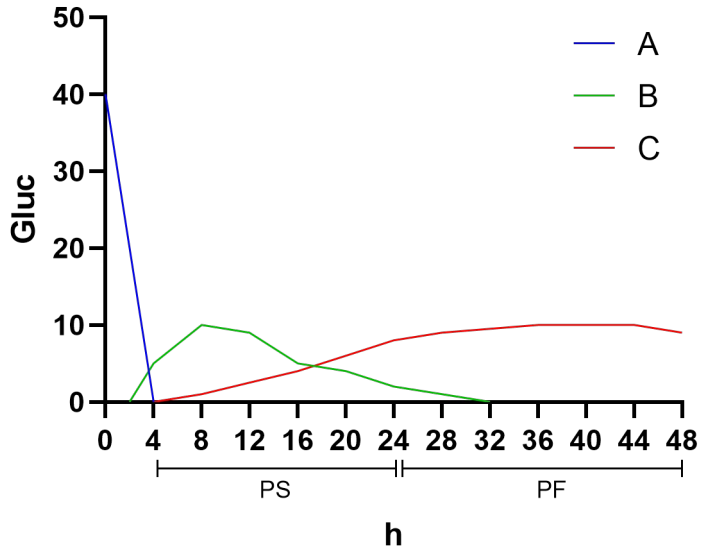


Figure 1 Usage of glucose during different phases of starvation. A, B and C indicate different sources of glucose. Gluc – glucose used (g/h); h – time in hours; PS – postabsorptive state: PF – prolonged fasting.

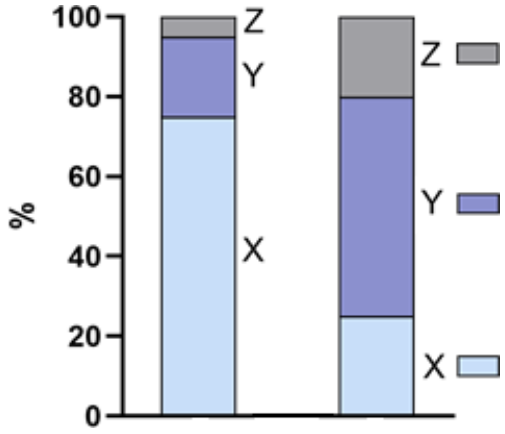


Figure 2 Percentage (%) of glucose production in different gluconeogenic organs. X, Y, Z correspond to these different gluconeogenic organs.

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. On figure 1, A corresponds to gluconeogenesis, B to glycogen and C to exogenous source.
B. On figure 1, A corresponds to exogenous source, B to glycogen and C to gluconeogenesis.
C. On figure 2, X corresponds to kidney, Y to liver and Z to intestine
D. Glycogen is an important source for glucose synthesis that can be found primarily in the liver and adipose tissue.

Q28

In the motor end plate, there is a type of rapid chemical transmission, mediated by the neurotransmitter acetylcholine (ACh). ACh vesicles are concentrated in the area of the motor nerve closest to the muscle, the active zone, and are then released (via exocytosis) into the synaptic cleft. Botulinum toxin has a great impact at motor end plate and Ach-mediated chemical transmission.

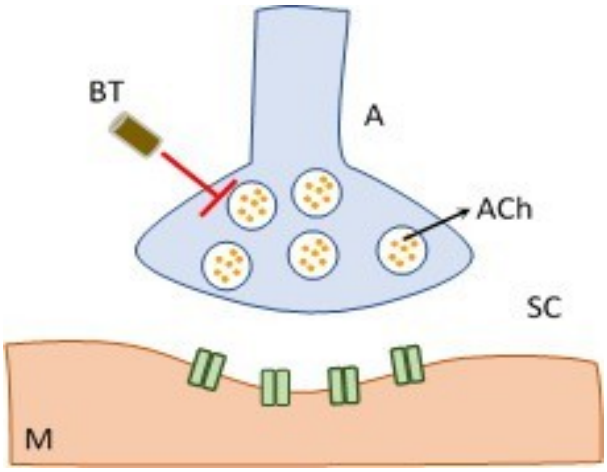


Figure 1 Motor end plate. Impact of Botulinum toxin. BT - Botulinum toxin; M – muscle; SC – synaptic cleft; Ach – acetylcholine; A – motor axon.

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. Botulinum toxin blocks the release of ACh

B. Botulinum toxin promotes constant muscle contraction.

C. Botox, the alternative name of botulinum toxin is produced by the fungus

Clostridium botulinum

D. Under steady-state conditions Ach release from the motor axon allows

action potential.

Q29

Thermogenesis is the process by which organisms produce heat. Heat production is mediated by uncoupling protein 1 (UCP1), which uncouple the proton gradient formation of electron transport chain from ATP production, in oxidative phosphorylation.

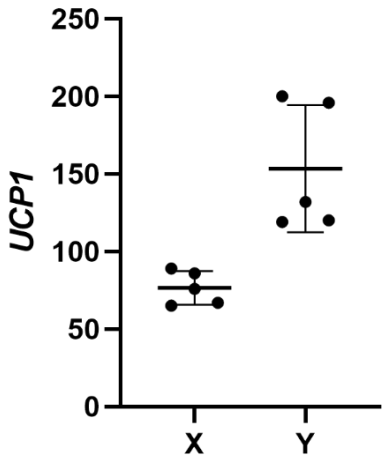


Figure 1 mRNA expression of UCP1 gene comparing two different polymorphisms (X and Y).

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. On figure 1, the expression of *UCP1* could relate to polymorphisms found in European (X) and Arctic (Y) populations
B. An increase in *UCP1* expression can be an adaptation to regions with high environmental temperatures.
C. Non-shivering thermogenesis occurs primarily in white adipose tissue
D. In humans, brown fat progressively reduces from newborn to adults.

Q 30

Figure 1 shows histological section of the thin skin is depicted, where the different tissues that constitute the skin can be observed. (1) is the external part, which is exposed to the air.

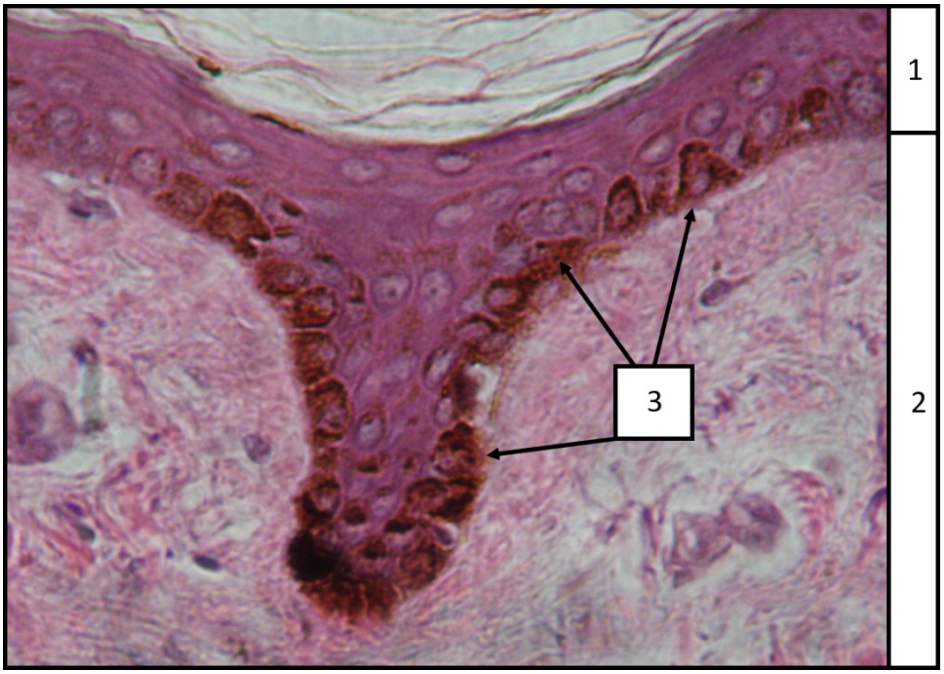


Figure 1. Histological section of the human thin skin. Mixed haematoxylin (purple basic dye) and eosin (pink acid dye) staining.

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. (1) and (2) are, respectively, the dermis and the epidermis
B. In mammals, (1) is a dry, keratinized, tissue that prevents water loss from the body as an adaptation to terrestrial life.
C. (3) are cells that accumulate a pigment in order to protect the basic layer of tissue (1), where the germinal (stem) cells are present, against the deleterious action of ultra-violet radiation
D. (3) are cells that accumulate a hormone called melatonin.

Q 31

The following figure depicts a histological section that represents a physiological system where two types of cells interact. Striated skeletal muscular fibers of the mammalian body contract under voluntary decision.

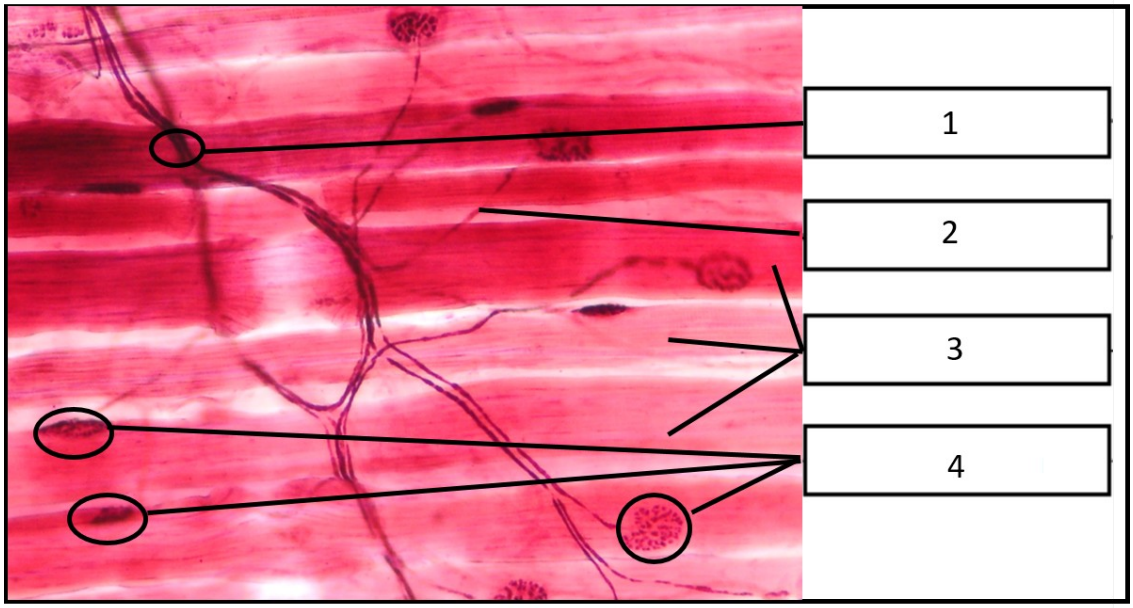


Figure 1. Histological section. (1) Nerve bundle (2) Individual axons (3) Muscle fibers (4) neuromuscular junctions. Striated skeletal muscle is stained in pink and the dark staining is the result of acetylcholinesterase activity.

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. Individual axons use acetylcholine as neurotransmitter. A single axon can innervate several fibers and give rise to a coarse and uniform movement of several fibers in the muscle while a “one axon-one fiber” type of neuromuscular junction gives rise to a very fine-tuned and precise movement of individual fibers within the muscle.

B. Individual axons use adrenaline as neurotransmitter. A single axon can innervate several fibers and give rise to a coarse and uniform movement of several fibers in the muscle while a “one axon-one fiber” type of neuromuscular junction gives rise to a very fine-tuned and precise movement of individual fibers within the muscle.

C. Individual axons use acetylcholinesterase as neurotransmitter. A single axon can innervate several fibers and give rise to a very fine-tuned and precise movement of individual fibers within the muscle while a “one axon-one fiber” type of neuromuscular junction gives rise to a coarse and uniform movement of several fibers in the muscle

D. Individual axons use serotonin as neurotransmitter. A single axon can innervate several fibers and give rise to a very fine-tuned and precise movement of individual fibers within the muscle while a “one axon-one fiber” type of neuromuscular junction gives rise to a coarse and uniform movement of several fibers in the muscle

Q 32

The following figure is a scheme that represents the Vertebrate spinal cord and the three connective tissue membranes (4) that ensheath it.

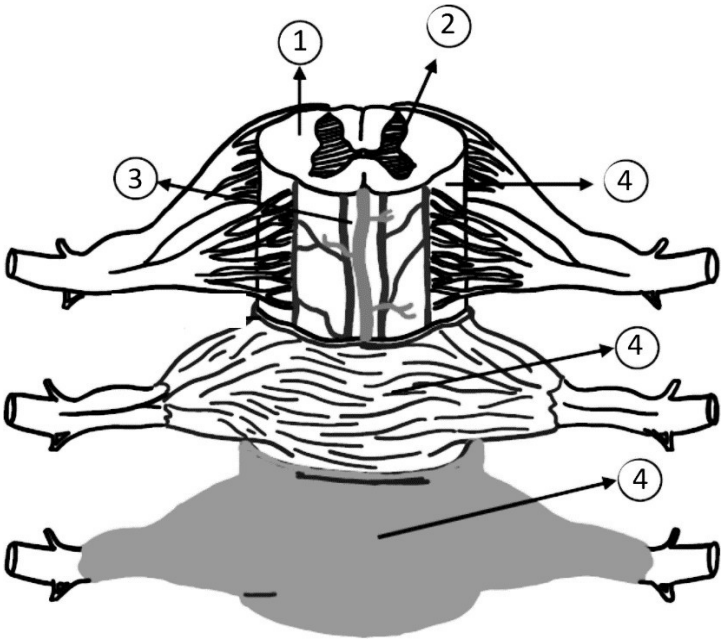


Figure 1. Schematic representation of the spinal cord.

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. (1) is the white matter, which consists of neuronal cell bodies and glial cells such as astrocytes and oligodendrocytes

B. The white matter (1) and the grey matter (2) are in the same relative position to one another in the brain, when compared to the spinal cord: (2) inside and (1) outside, closer to the meninges.

C. (4) are known as the meninges and are named pia-mater, arachnoid and dura- mater.
D. (3) are blood vessels (arteries and veins), located between the arachnoid and the pia-mater meninges, that irrigate the spinal cord nervous tissue.

Q 33

Organoids are three-dimensional culture models where cells grow and differentiate into several cell types, allowing a partial reconstitution of a complex cellular organisation where organotypic interactions can take place and so mimicking some of the functions of the respective *in vivo* organ. Providing the proper stimuli, such as culture medium supplements, appropriate growth and differentiation factors, hormones, etc, as well as an adequate physical scaffold, tissues can self-organize to a certain extent and generate organoids that have proven very useful in basic and applied biomedical research. One of the examples of organoids that have already been successfully produced in several laboratories are brain organoids (the so-called “mini- brains”).

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. Using organoids made from cells harvested from a specific donor carrying a particular disease would make them especially useful for studying the disease progression and the personalized use of therapeutic agents in that particular patient.
B. Organoids are safe biological materials to be transplanted in human patients.
C. Entire organs (a whole kidney or liver, for instance) can easily be grown *in vitro* for the purpose of transplantation into patients, with no limitations, making organoids a useless technology.
D. “Mini-brains” are appropriate models to study complex behaviours *in vitro*.

IV. Ethology

Q34

Cicadas are singing insects whose presence is easily detected by the male-calling song during the mating season. **Figure 1** shows species of the genus *Tettigettalna*, a group of small-sized cicadas from southern Europe with similar morphology but distinct male calling songs. They have overlapping distributions, being often found in sympatry, suggesting an important role for acoustic behaviour in their diversification and maintenance of reproductive isolation between sister species. In fact, results from acoustic, morphometric and genetic analyses showed that *T. mariae* and *T. argentata* from the southernmost locations exhibit significant acoustic and morphological variation without genetic differentiation. These results may point to different scenarios. Statements are specifically about *T. mariae* and *T. argentata*.

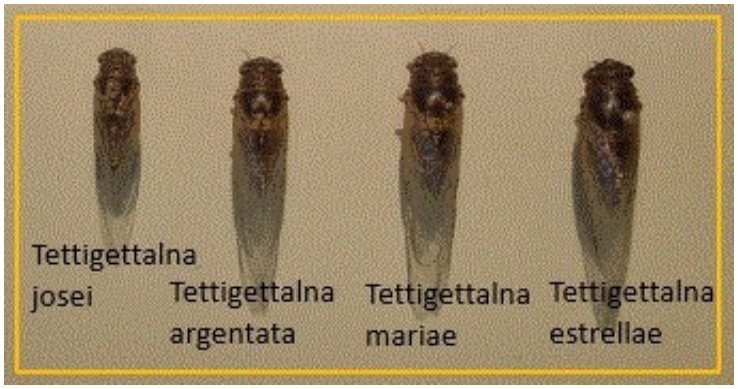


Figure 1 – Several cicada species from genus *Tettigettalna*

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. A past introgression episode between the two species

B. There is no gene flow between these sibling species, but they still retain ancestral polymorphism.

C. *T. mariae* haplotypes are only found in *T. argentata* specimens sampled in sympatry or allopatric locations within predicted past distribution of *T. mariae.* Since these haplotypes are not present in the majority of *T. argentata* distribution area, they are likely to belong to the original gene pool of *T. argentata*.

D. Divergence in acoustic behaviour probably restricts gene flow in sympatry.

Q35

Cicada orni is one of the most abundant and common cicadas in Mediterranean areas of southern Europe. Males of the species show intense acoustic behaviour. The analyses of the structure of the calling song in *C. orni* over a wide distribution range of the species in the Mediterranean region was performed to better understand the pattern of its geographic variation. **Figure 1** shows some of the studied acoustic variables.

These acoustic variables revealed some variation among specimens from different regions, such as shown by in **Figure 2.**

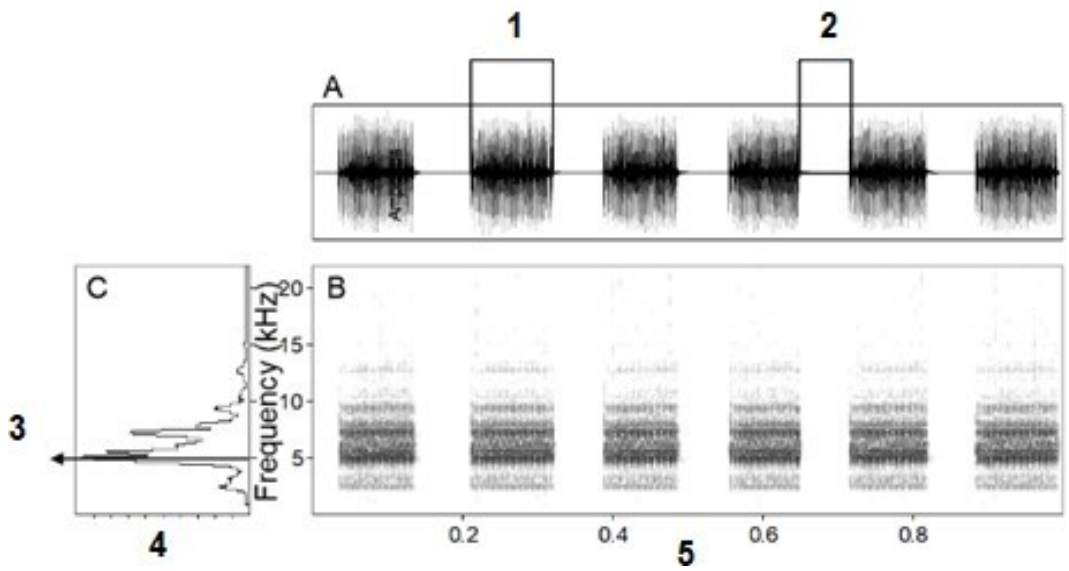


Figure 1. Calling song of *C. orni* **A**. Oscillogram (amplitude vs time); **B.** Sonogram (frequency vs time); **C**. Mean amplitude spectrum (frequency vs amplitude). **1**. Echeme duration (s); **2**. Inter-echeme interval (s); **3**. Peak frequency (Hz). **4.** Intensity (V) **5.** Time

(s). The echemes are composed by groups of pulses, and the duration of the interval between them is the inter-echeme.

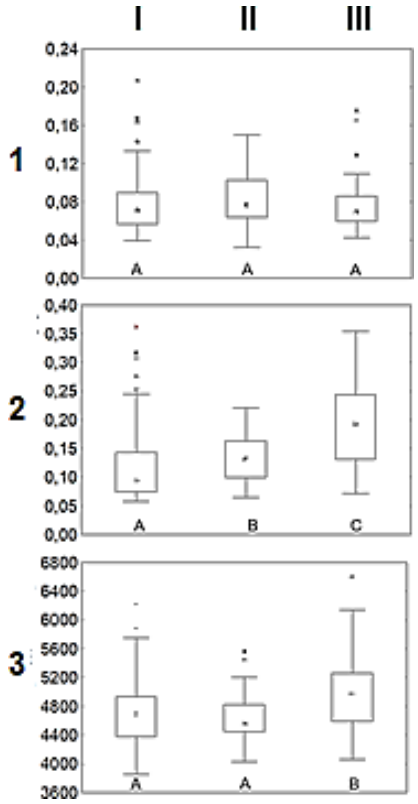


Figure 2. Boxplots of three acoustic variables of the calling song of *C.orni* in (**I**) Iberian Peninsula, (**II**) France and (**III**) Greece. Regions with significant differences (Mann- Whitney, p«0.05) share no letters. **1**. Echeme duration (s); **2**. Inter-echeme interval (s);

3. Peak frequency (Hz). The echemes are composed by groups of pulses, and the duration of the interval between them is the inter-echeme.

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

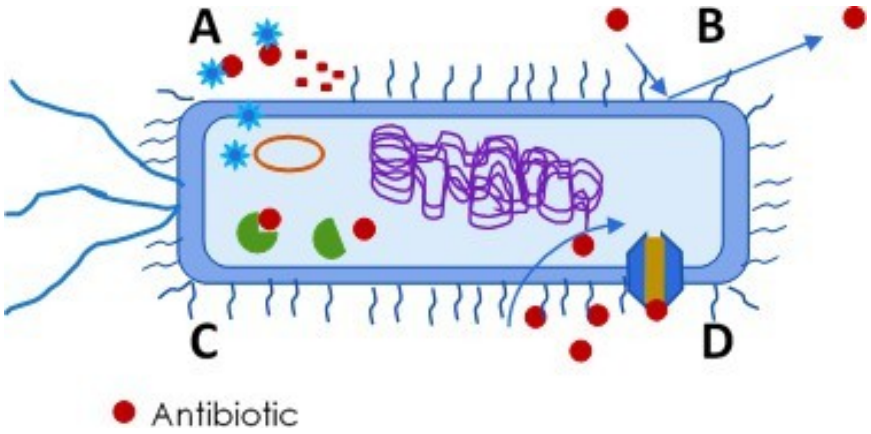
A. Echeme duration is not important in species recognition.
B. Inter-echeme interval is probably not important in species recognition.
C. Iberian Peninsula appears as the most differentiated area.
D. Differentiation in Greek specimens might have been the result of strong geographic isolation.

V. Genetics and Evolution

Q36

Antibiotic resistance is a constant Public Health problem as more antibiotics are rendered ineffective by drug-resistant bacteria which have developed resistant mechanisms to conventional antibiotics.

Bacteria can resist antibiotics via different resistance mechanisms. In the **figure below**, identify the mechanisms **A – D** from the list **1 – 6**: (*4 points, 1 point each correct answer*)



1. Modification of the antimicrobial drug target.
2. Production of hydrolytic enzymes, such as β-lactamases, that inactivate the surrounding antimicrobial drug.
3. Production of bacteriocins that destroy the antibiotic.
4. Reduction of the antimicrobial drug concentration in the intracellular environment by pumping them out before they can take effect.
5. Antibiotic degradation inside the cell
6. Natural resistance due to an unusually impermeable cell membrane or lack of the target recognized by the antibiotic

Q37

Concerning the mechanisms of action of antibiotics and the molecular mechanisms responsible for antibiotic resistance and spread.

Indicate whether each of the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. Antibiotic resistance can be acquired through mutations in the bacterial DNA.
B. The presence of antibiotics is thought to play an important role in antibiotic resistance (selective pressure). This suggests a Lamarckian mechanism of adaptive evolution.
C. Plasmids, belonging to a class of genetic elements known as mobile genetic elements , can transfer antibiotic resistance genes when moving from bacterial cells to eukaryotic cells.
D. Chloramphenicol is a bacteriostatic antibiotic that inhibits the peptidyl transferase activity of the bacterial ribosome (responsible for peptide bond). This directly prevents transcription.

Q38

Advances within biotechnology, genetic engineering, and synthetic chemistry have opened new possibilities towards the search for antimicrobial treatment of bacterial infections, which can substitute for antibiotics. Some are currently undergoing clinical trials, while others are in different stages of research. Some will work by themselves, while others exhibit the potential to act synergistically when combined with certain chemical antibiotics already available on the market.

Match the new antimicrobial therapies on the left (1-6) with the advantages mentioned on the right side of the table (A-F) below:

(*6 points, 1 point each correct answer*)

New promising antimicrobial therapies	**Advantages**
1. Antibodies	A. Silences gene expression of resistance genes by interfering with their transcription.
2. Probiotics	B. Safe method, because do not possess any affinity whatsoever with eukaryotic cells.
3. Bacteriophages /phage therapy	C. Enhances the human microbiota. When consumed or applied to the body may bring health benefits.
4. Antimicrobial peptides	D. Specificity and the inability of bacteria to develop resistance against them.
5. Lysin therapy	E. Causes disruption of the bacterial membrane and can be used in the treatment of bacterial, fungal and viral infections, as well as in the prevention of biofilm formation
6. Antibacterial oligonucleotides	F. Targets one of the five bonds in peptidoglycan (murein) and amounts in the order of nanograms cause immediate lysis of the target Gram-positive bacterial cells.

Q39

SARS-CoV-2 is a coronavirus (CoV) that emerged in 2019 and is responsible for the COVID-19 disease in humans. Other coronaviruses affecting humans are the SARS- CoV and MERS-CoV, responsible for the previous epidemics Severe Acute Respiratory Syndrome in 2002, and Middle East Respiratory Syndrome in 2012. Transmission of coronavirus to humans has likely occurred through civets for SARS-CoV and camels for MERS-CoV. The origin of SARS-CoV-2 is under intense debate. Studies based on full- genome CoV variants isolated from bat (Bt), pangolin (Pn), camel (Cm), civet (Cv), pig (Pi) and humans (Hu) have provided information on the ancestry and evolution of coronaviruses. Several authors suggest a zoonotic origin and different hypotheses have

been proposed about the last animal intermediate before transmission to humans using phylogenetics.

Figure 1 below shows an inferred phylogeny from the ORF1ab genomic region of coronavirus. The prefixes of virus names indicate the host species. The strains A (isolated from bat), B (isolated from pangolin) and C (SARS-CoV isolated from a human in 2003) are also represented in Figure 1 in Q40. HuCoV2 WH01 represents the human SARS-CoV-2 isolated from Wuhan, China in 2020.

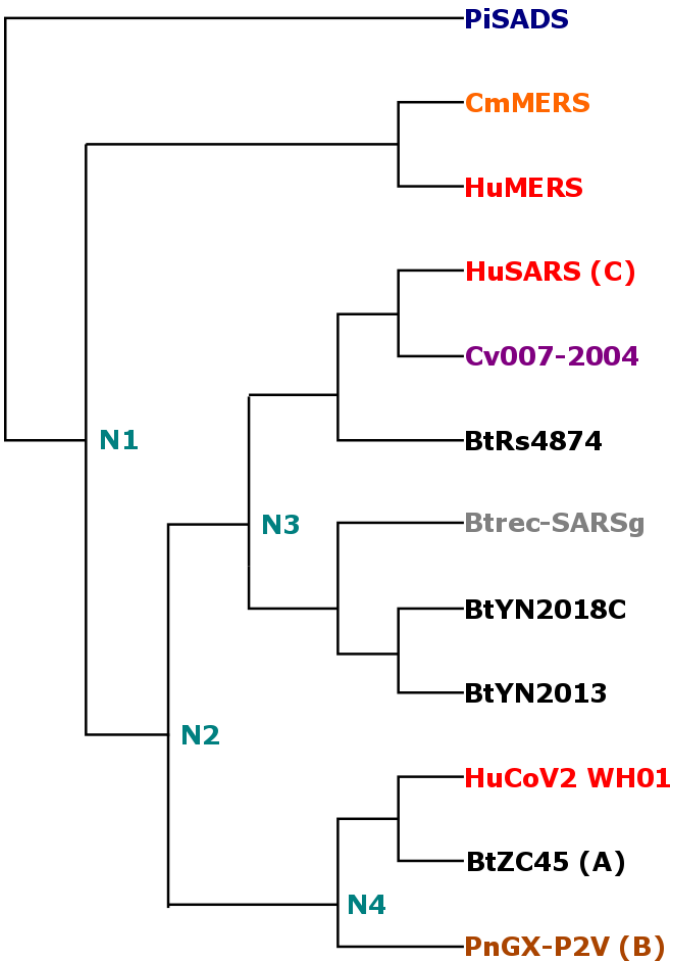


Figure 1. Topology of the phylogenetic tree inferred from the ORF1ab genomic region. Coronavirus analysed were isolated from different hosts (represented by different colors in the tree): Bat (Bt): BtRs4874, BtYN2018C, BtYN2013, BtZC45 (**A in Fig.1 from Q40**); lab recombinant (bat+cell culture): Btrec-SARSg; Pangolin: PnGX-P2V (**B in Fig. 1 from Q40**); Civet (Cv): Cv007-2004; Camel (Cm): CmMERS; Pig (Pi) PiSADS; Human CoV variants (Hu): HuSARS (isolated in from a patient in 2003, **C in Fig.1 from Q40**), HuMERS, HuCoV2. Data from Sallard *et al.* 2021.

Based on the inferred phylogeny, indicate whether the following statements are true

(T) or false (F). (*4 points, 1 point each correct answer*)

A. The most recent common ancestor of human SARS-CoV and SARS-CoV-2 (HuCoV2) is N2.
B. The lineage most closely related to the human SARS-CoV-2 (HuCoV2) is the bat isolate BtYN2013.
C. The Pig (PiSADS) lineage gave rise to all other coronaviruses shown in the phylogenetic tree
D. The recombinant bat isolate (Btrec-SARSsg) obtained from cell culture in the laboratory is not directly related to SARS-Cov-2, as descendent lineages from their common ancestor include other isolates.

Q40

The coronavirus genome is about 30,000 nucleotides, organized in ten genes (represented at the top in **Figure 1** below), including the ORF1ab, coding for a polyprotein precursor involved in replication, and the S gene coding for the spike protein that recognizes the receptors of the host cells. Profiles of Percent of Identical Positions (PIP) reveal the percentage of sites that are identical between two genomes. **Figure 1** below shows PIPs produced from full-genome alignments between the SARS-CoV-2 and three other coronaviruses (**A**: bat CoV isolate; **B**: pangolin CoV isolate; and **C**: human SARS-CoV isolated in 2003).

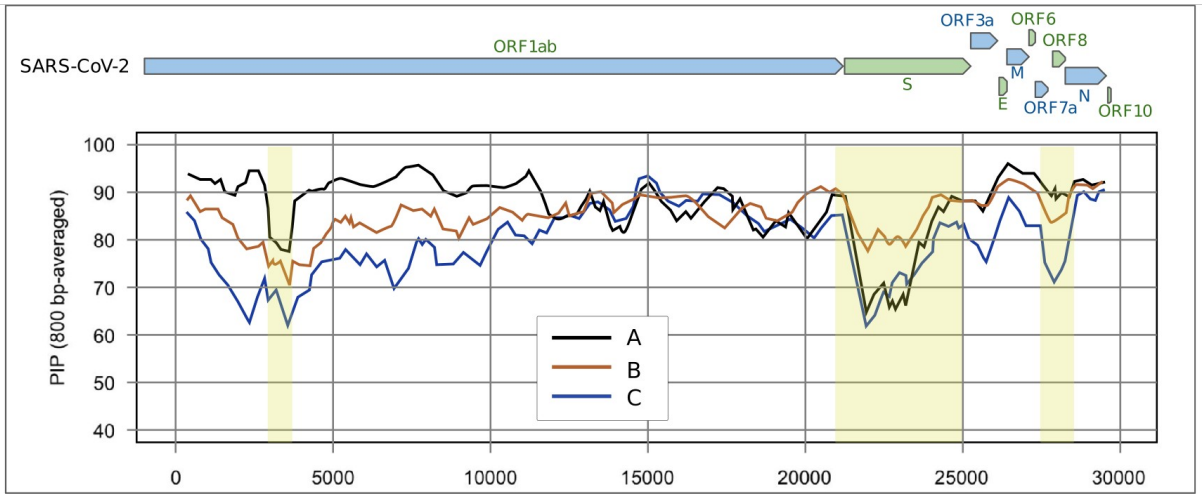


Figure 1. Profiles of Identical Positions (PIP) along the genome between human SARS- CoV-2 and other coronavirus sequences isolated from bat (A), pangolin (B) and human SARS-CoV isolated in 2003 (C).

Based only on PIP results from Figure 1 indicate whether the following statements are true (T) or false (F). (*4 points, 1 point each correct answer*)

A. The fact that important genomic regions in the infection/viral replication process (highlighted in yellow) have lower PIP indicates that SARS-CoV-2 originated from SARS-CoV.
B. The genetic similarity in the S region between pangolin and human SARS-CoV-2 is lower than that between bat and human SARS-CoV-2. Given the importance of the S region on the cell infection process, this shows that the last animal host before transmission to humans was a bat.
C. Recombination between coronavirus infecting bats and pangolins can explain that a pangolin isolate is closer to SARS-CoV-2 at protein S whereas a bat isolate is closer to SARS-CoV-2 in the rest of the genome
D. The phylogenetic relationship between the pangolin isolate and HuCoV2 would be different at the S gene than at the rest of genome.

Q41

Knowing that a population of a diploid organism is at Hardy-Weinberg equilibrium for a certain locus with 5 alleles (a1, a2, a3, a4, a5) and knowing that:

- the relative frequencies of certain alleles are: a1 = 0.2, a3 = 0.2, a4 = 0.2;
- the relative frequency of the heterozygotic a4a5 is 0.04;
- the homozygotic a2a2 is 0.09.

Indicate whether each of the following statements is true (T) or false (F). (*5 points, 1 point each correct answer*)

A. The frequency of the allele a2 is 0.03
B. The frequency of the allele a2 is 0.3
C. The sum of the relative frequency of the five alleles is 1
D. The sum of the relative frequency of the five alleles is 2
E. The frequency of the allele a5 is 0.1

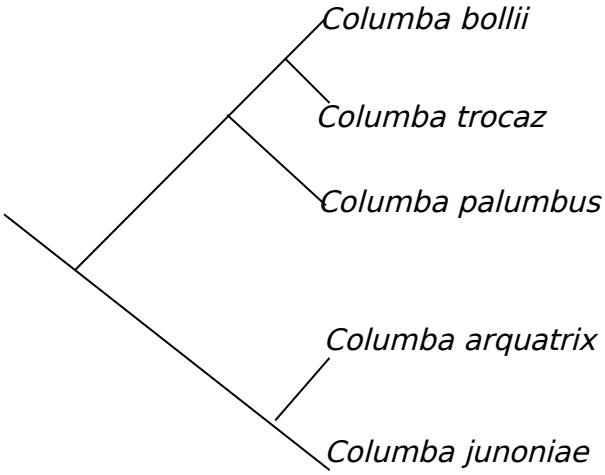
Q42

Based on several mitochondrial DNA genes it is possible to obtain the phylogenetic relationship among the five bird species of the genus *Columba*. Samples from the species *Columba junoniae* and *Columba bollii* are from the Canary Islands, *Columba trocaz* from Madeira Island, *Columba palumbus* from Europe (mainland) and *Columba arquatrix* from Africa.

A ssuming that the cladogram shown represents the most parsimonious phylogeny:

Indicate whether each of the following statements is true (T) or false (F).

(*5 points, 1 point each correct answer*)



Cladogram representing the phylogenetic relationship among the five bird species of the genus *Columba.*

A. The two Canary Island species are phylogenetically close to each other than they are to any other species of the cladogram
B. One of the Canary Islands species is phylogenetically close to the Madeira Island species than they are to any other species of the cladogram
C. The European and the African species are in the same clade and are phylogenetically close to each other than they are to any other species of the cladogram
D. The two Canary Island species have two different origins
E. The Madeira Island species is phylogenetically close to the African species than they are to any other species of the cladogram

Q43

Several codons of the mitochondrial DNA *cytochrome b* gene are shown.

Based on DNA sequences and the Vertebrate mitochondrial DNA code:

Indicate which sentences are true (T) or false (F).

(*5 points, 1 point each correct answer*)

Columba junoniae	AAC GGG ATA CGT ACA GAT ATC ATT CCC CTA TAT CGG CCA CCC CTT ACA
Columba arquatrix	AAC AGG ATA CGT GCA AAT ATC ATT CCC CTA CAT CGG CCA TCC CTT ACA
Columba trocaz	GAC GGG ATA TGT ACA AAT ATC GTT CCC ATA CAT CGG CCA TCC CTT ACA
Columba palumbus	GAC AGG ATA TGT ACA AAT ATC GTT CCC ATA CAT CGG TCA CCC CTT ACA
Columba bollii	AAC GGG ATA TGT ACA AAT ATC GTT TCC ATA CAT CGG CCA TCC CTT GCA

Partial sequence of the mitochondrial DNA *cytochrome b* gene

The Vertebrate Mitochondrial Code (** Indicate a stop codon)*

TTT F Phe	TCT S Ser	TAT Y Tyr	TGT C Cys
TTC F Phe	TCC S Ser	TAC Y Tyr	TGC C Cys
TTA L Leu	TCA S Ser	TAA * Ter	TGA W Trp
TTG L Leu	TCG S Ser	TAG * Ter	TGG W Trp
CTT L Leu	CCT P Pro	CAT H His	CGT R Arg
CTC L Leu	CCC P Pro	CAC H His	CGC R Arg
CTA L Leu	CCA P Pro	CAA Q Gln	CGA R Arg
CTG L Leu	CCG P Pro	CAG Q Gln	CGG R Arg
ATT I Ile	ACT T Thr	AAT N Asn	AGT S Ser
ATC I Ile	ACC T Thr	AAC N Asn	AGC S Ser
ATA M Met	ACA T Thr	AAA K Lys	AGA * Ter
ATG M Met	ACG T Thr	AAG K Lys	AGG * Ter GCT
GTT V Val	A Ala	GAT D Asp	GGT G Gly GCC A
GTC V Val	Ala	GAC D Asp	GGC G Gly GCA A Ala
GTA V Val	GAA E Glu	GGA G Gly	
GTG V Val	GCG A Ala	GAG E Glu	GGG G Gly

A. There are no amino-acid substitutions between species *Columba junoniae* and

   Columba bolli in the codons shown.

B. There are two codon substitutions between species *Columba trocaz* and

   Columba palumbus in the codons shown

C. One amino-acid substitution from *Columba arquatrix* to *Columba*

   junoniae , in the codons shown, is from Ala to Thr

D. There are 8 amino-acid substitutions between species *Columba junoniae* and

   Columba bolli in the codons shown

E. There are 18 amino-acid substitutions between species *Columba junoniae* and

   Columba bolli in the codons shown

Q44

The ABBA-BABA test is a commonly used approach in evolutionary biology to identify admixture (cases where two distinct populations interbreed) using genetic data. It involves the comparison of four populations or species (we will call them “groups” henceforth), whose phylogenetic relatedness can be represented as a tree (Figure 1), where X and Y are two closely related groups, W is a group that could have admixed with X or Y, and Z is an outgroup.

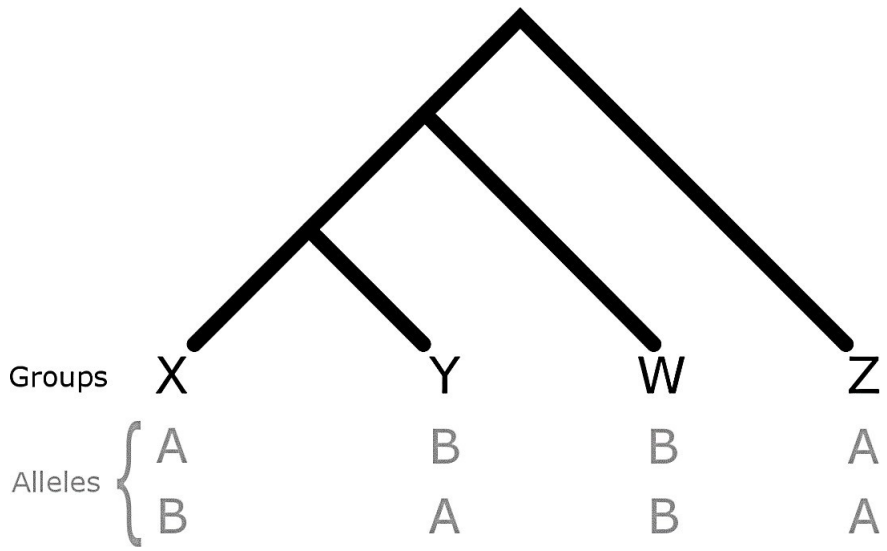


Figure 1. Phylogenetic relationship between four groups X, Y, W, Z and representation of ABBA and BABA allelic patterns across the four groups.

The ABBA-BABA test focuses on single nucleotide polymorphisms sites in the genome that have two alleles: A and B. It is possible to detect admixture considering two possible allelic patterns across the four groups:

- "ABBA" sites: where group X has the allele A, Y has the allele B, W has the allele B, Z has the allele A;
- "BABA" sites: where group X has the allele B, Y has the allele A, W has the allele B, Z has the allele A.

The *D* statistic is defined as (#BABA - #ABBA) / (#BABA + #ABBA), where #BABA is the number of BABA sites and #ABBA is the number of ABBA sites.

Imagine that a team of researchers sequenced the genomes of two modern humans from Kenya (X) and China (Y), one Neanderthal (W) and one Chimpanzee (Z), using ABBA-BABA to test if there was admixture between modern humans and Neanderthal. The researchers have identified 10,827 "ABBA" sites and 9,867 "BABA" sites.

Indicate whether each of the following statements is true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. If there is ***no* admixture**, we expect a nearly equal number of ABBA and BABA sites across the genome
B. There is evidence of admixture between the Kenyan population and Neanderthal
C. The observed excess of ABBA sites (compared to BABA) could be explained by a structured ancestral population (of X, Y and W) such that the Neanderthal lineage was closer to the ancestral of Chinese
D. The ABBA–BABA test allows testing for admixture between Kenyan and Chinese modern human populations

Q45

Toehold switches are a class of synthetic RNA regulators that can repress gene expression by sequestering the Ribosome Binding Site (RBS) in a stable hairpin structure. Upon the addition of a small RNA called the toehold trigger, the hairpin structure unfolds, and the RBS is released.

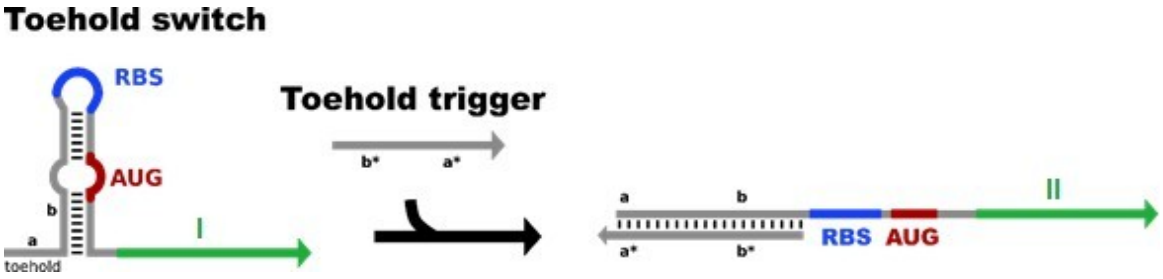


Figure 1. Toehold switch. I. Repressed gene; II. Activated gene.

Image credits: Jacob Mejlsted, Own drawing. *Inspired by* Green *et al.* (2014)

Indicate whether the following statements are true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. Toehold switches work by repressing the translation of the gene
B. A strong hairpin structure can be made from direct repeats in the RNA sequence
C. The toehold switch will work just as well in eukaryotes as in bacteria
D. One toehold switch can control all genes in an operon

VI. Ecology

Q46

Salt marshes are complex coastal environments usually located within estuarine systems. Estuaries receive important inputs of pollutants as they are often situated in the vicinity of highly populated and industrialized areas. Once metal contaminants enter the salt marsh, they become distributed in sediments, pore water and plants, which are able to capture metals from the soil through their roots.

The uptake of metals by plants depends upon their availability in sediments, which is determined by the physical and chemical characteristics of the sediments. The activity of plants can alter these factors inducing variations in the speciation and availability of metals. Differences in metal accumulation levels occur between plant species and also between sediments colonized by different plant species.

FIG. 1

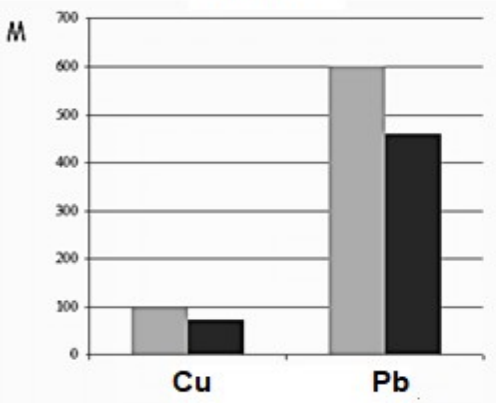


FIG. 2

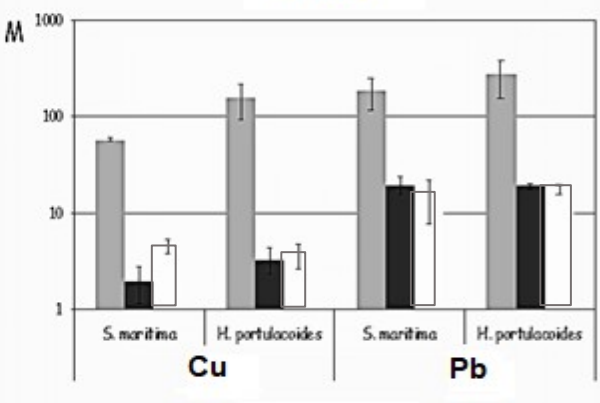


Figure 1 - M = Metal concentration (µg g^{-1} dry weight) of copper (Cu) and lead (Pb) in salt marsh sediments colonized by *Spartina maritima* (gray) and *Halimione portulacoides* (black).

Figure 2 - M = Metal concentration (µg g^{-1} dry weight) in roots (grey), stems (black) and leaves (white) of *Spartina maritima* and *Halimione portulacoides.*

Indicate whether each of the following statements is true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. Salt marshes dominated by *S. maritima* may have a stronger capacity for the retention and phytostabilisation of Cu and Pb than those dominated by *H. portulacoides*

B. Salt marshes mainly colonised by *H. portulacoides* can translocate more metals above ground , sourcing these metals to the marsh ecosystem

C. The use of these species for phytoremediaton of polluted marshes could be applicable from two different perspectives: phytostabilisation of metals in sediments, or phytoextraction by accumulation in aboveground plant tissues for subsequent plant removal.

D. *S.maritima* seems to be a more appropriate plant to use, for phytoextraction purposes

Q47

Topography creates contrasting microclimates, especially between northern and southern slopes in drylands, which result in clear differences of tree cover patterns in the landscape. In a study performed in a holm oak community (a key tree species in Mediterranean drylands) the impact of microclimatic conditions on holm oak trees regeneration success was studied using Potential Solar Radiation (PSR) as an integrated measure of microclimatic conditions.

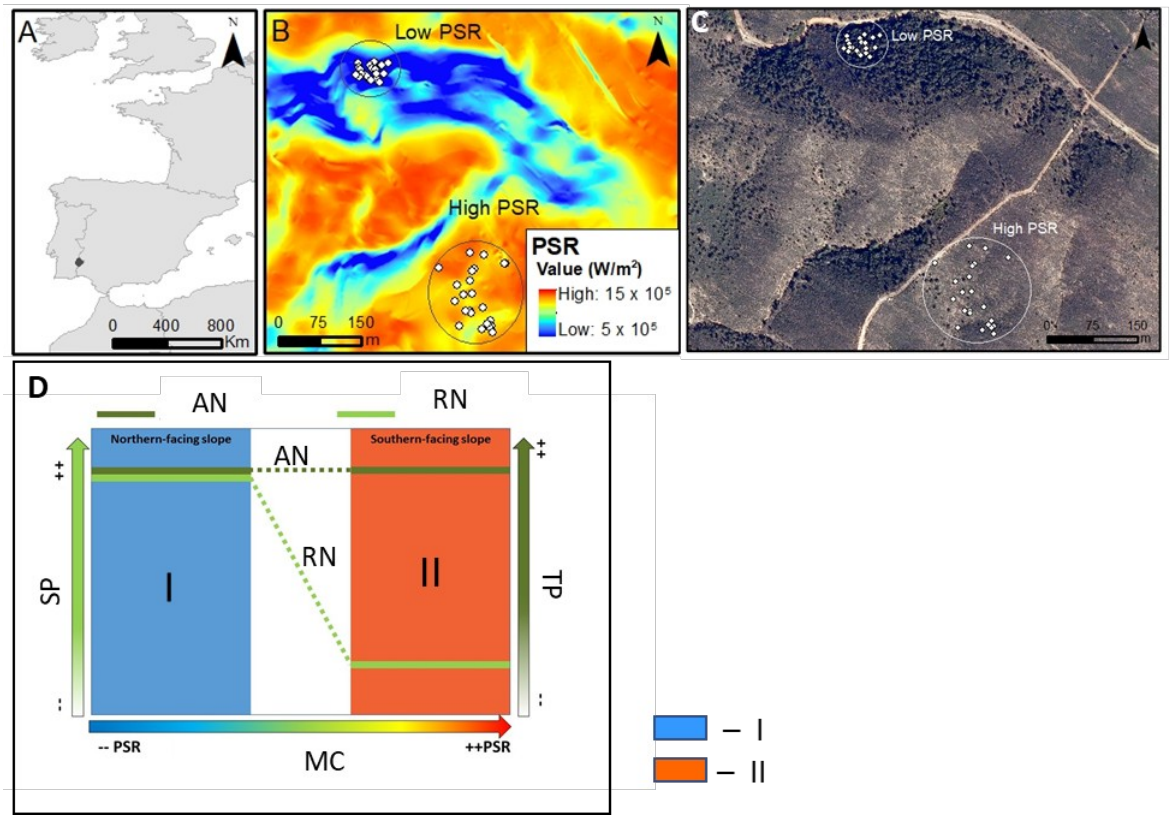


Figure 1- Location of the study area in Europe (A; dark circle) and sampling area with white points representing the trees sampled in high and low values of Potential Solar Radiation (PSR) (B) and with a 2010 background aerial photo (C) and a conceptual

diagram of tree performance along contrasting microclimatic conditions for different tree stages (D): tree (adult niche - **AN**) and seedlings (regeneration niche - **RN**) in a PSR gradient (**MC** – microclimatic conditions) **SP** - seedling performance, **TP** - tree performance. **I** – Northern-facing slope; **II** – Southern-facing slope.

Indicate whether each of the following statements is true (T) or false (F). (*4 points, 1 point each correct answer*)

A. In the northern hemisphere, the microclimate conditions range from warm and dry (xeric) on northern slopes to cool and moist (mesic) on southern slopes
B. These findings suggest that on northern slopes, natural regeneration is higher with established trees facilitating the germination and growth of new trees
C. Microclimate, as measured by the PSR, was the key driver in determining the natural regeneration and tree performance of holm oak on a local scale
D. The width of the regeneration niche is expected to be narrower than that of adult trees, since their water sources are restricted to soil water content especially under more stressful microclimatic conditions

Q48

Figure 1 shows the results obtained in a deforestation program in a hydrographic basin in two experimental plots. Control plot without any deforestation intervention (**P1**) and plot 2 (**P2**) where the forest (1-60 years old forest) was cut (arrow), and herbicide application prevented the vegetation development for the 3 following years (2 - succession supressed). After this time, herbicide was no longer applied, allowing vegetation to evolve naturally (3 -succession). (Plot 1 – dark circles, Plot 2 - red circles),

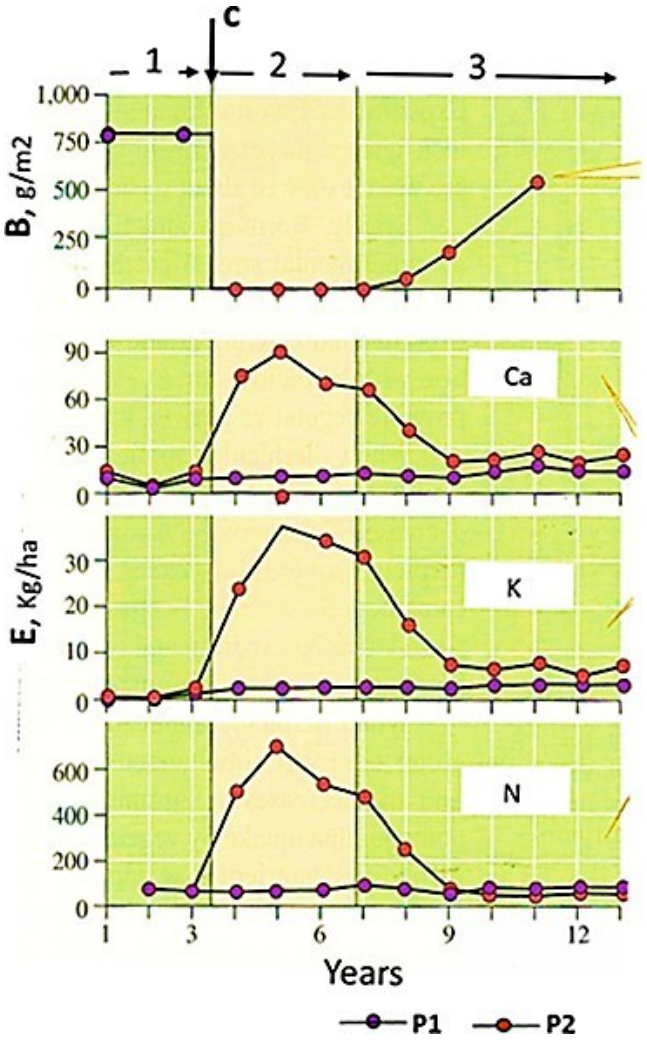


Figure 1. Periods: **1** – 60 years old forest, **2** - succession supressed, **3** – succession; **C** (arrow) – forest clear cut, **B** – plant biomass (g m^{-2}), **E** - annual net export (Kg ha^{-1}) for **Ca** – calcium, **K** -potassium, **N** – nitrogen . **P1** – Plot 1, **P2** – Plot 2

Indicate whether each of the following statements is true (T) or false (F). *4 points, 1 point each correct answer*)

A. After the suppression of the herbicide application, a primary succession starts with a gradual increase in biomass
B. After cutting the vegetation and during herbicide application there is a marked increase in leaching of nutrients out of the system, contributing to a gradual depletion of these nutrients
C. During the 3-year period without vegetation cover, there is a change in the water balance of the system with a marked increase in evapotranspiration and runoff (streamflow)

D. This deforestation experiment showed that succession can reduce the loss of nutrients caused by disturbance

Q49

Figure 5 is a diagrammatic representation of sample plots (1 m^2) from two different herbaceous communities (**Community A** and **Community B**) containing the same six species but in different abundances. Each letter (a, b, c, d, e, f) represents a different species (Sp). The distribution of individuals among species is called species evenness or species equitability and is quite different between communities. Species diversity is a combination of richness and evenness. Here we use the Shannon-Wiener's index (H´) to calculate the diversity of the two communities (**Table 1**) where *s* is the total number of species, and ***pi*** is the proportion of all individuals in the sample that belongs to species *i*

$$H' = - \sum_{i=1}^{s} (pi). (\ln pi)$$

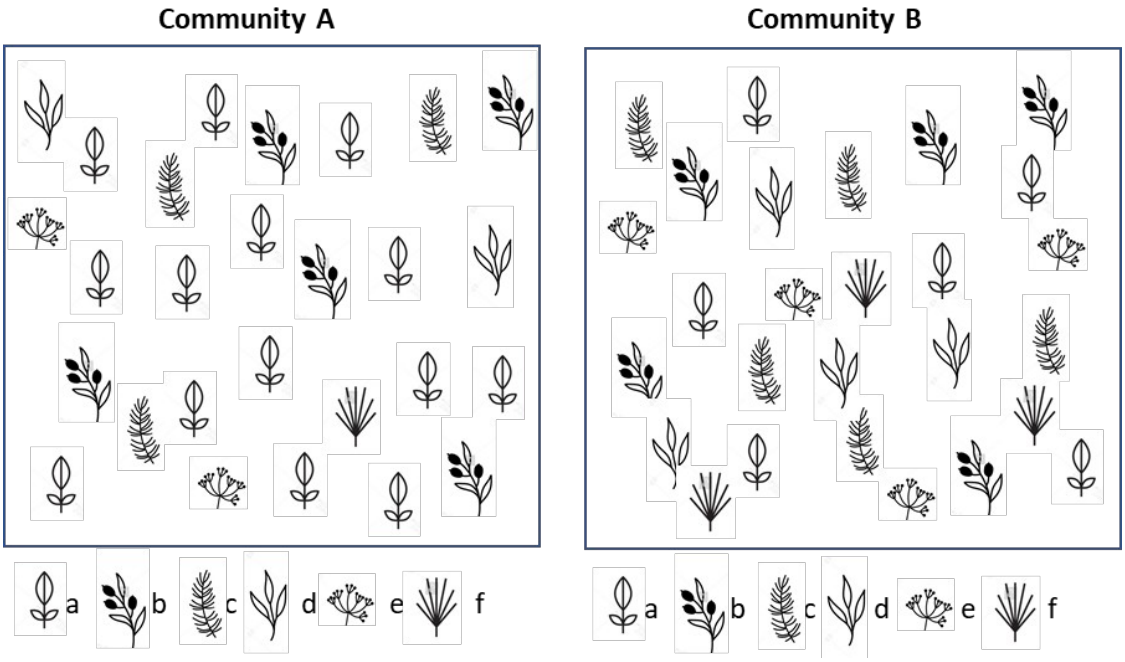


Figure 5 – Two different herbaceous Communities.

Table 1 – Calculating species diversity (H´) for two hypothetical herbaceous communities. Nº - number of individuals of different species

Com A					*Com B*				
Sp	*Nº*	*(pi)*	*ln pi*	*pi.lnpi*	*Sp*	*Nº*	*(pi)*	*ln pi*	*pi.lnpi*
a	14	0.56	-0.580	-0.325	a	6	0.24	-1.427	-0.343
b		0.20	-1.609	-0.322	b		0.20	-1.609	-0.322
c		0.12	-2.120	-0.254	c		0.20	-1.609	-0.322
d		0.08	-2.526	-0.202	d		0.16	-1.833	-0.293
e	2	0.08	-2.526	-0.202	e	4	0.16	-1.833	-0.293
f	1	0.04	-3.219	-0.129	f	3	0.12	-2.120	-0.254
Total					Total				

Indicate whether each of the following statements is true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. The total density (number of individuals m^{-2}) is the same in the two communities
B. The density of species b is higher in community A
C. The Shannon diversity index (H´) in community A is 1.827
D. Due to the dominance of one species in community A, Shannon-Wiener's diversity index is lower in this community

Q50

2021 is the International Year of Caves and Karst. The caves open real windows to the study of the vast subterranean ecosystem that hides under our feet. In a domain without light, the organisms that inhabit this environment evolve and adapt, showing convergent evolution similar to what occurs in the deep sea. What are the characteristics of cave- adapted animals?

Figure 1. Cave millipede, *Cylindroiulus villumi*, adapted to live in caves in Portugal, scale bar: 1 mm.

Indicate whether each of the following statements is true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. Are expected to live longer than their relatives at surface
B. Have a pronounced circadian rhythm
C. Are more diverse than surface organisms in their distribution areas
D. Have a high prevalence of endemic species

Q51

The salinization of groundwater is a global long-term environmental problem worldwide, exacerbated in arid and semi-arid regions by global warming. In a study, the dose- response curve and median lethal salt concentrations were estimated for three groundwater-adapted crustaceans (Cyclopoida, Harpacticoida and Syncarida) that live in aquifers of southern Australia (**Figure 1**).

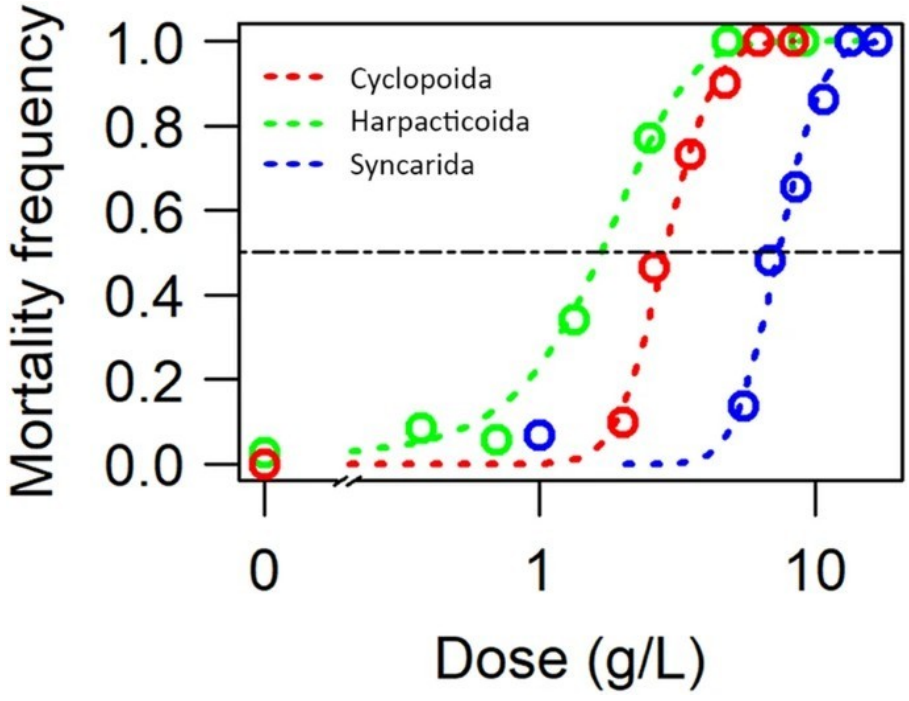


Figure 1 - Salt (NaCl) dose–response curves and median lethal concentrations (LC_{50}) at 96 hours. Y axis - Mortality frequency; X axis - Dose (g/L).

Indicate whether each of the following statements is true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. The crustaceans, Cyclopoida and Harpacticoida were less sensitive to salt than Syncarida
B. The presence of high concentrations of salt groundwater stresses the osmotic balance of the studied crustaceans
C. If we measure a maximum environmental concentration of salt (NaCl) of 10g/L in groundwater in the area of distribution of these species, they are endangered by salinization
D. If the temperature of groundwater increases, together with the salt concentrations, the effect on the groundwater crustaceans is likely to be magnified.

Biosystematics

Q52

Leeches are organisms belonging to the Phylum Annelida, Class Hirudinea. They are found predominantly in freshwater habitats, few are marine, and some are adapted to terrestrial life. As fluid feeders and bloodsuckers, leeches are more highly specialized than other Annelida. **Figure 1** presents a transversal section of a leech at the intestine level and the arrows point to glandular cells identified just under the epidermis. **What is their function?**

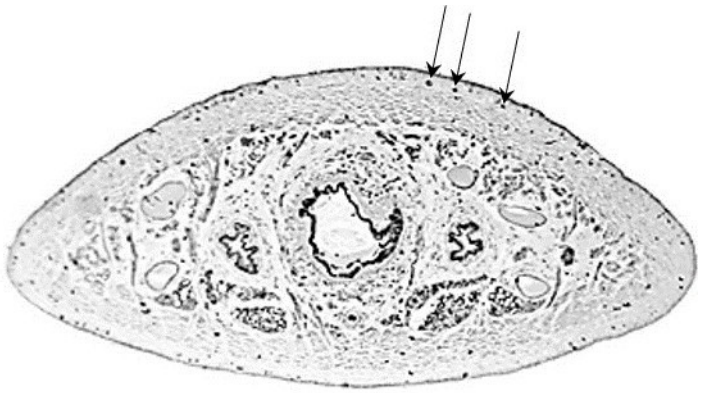


Figure 1. Transversal section of a leech at the intestine level.

Indicate whether each of the following statements is true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. Allow the organism to glide better in the habitat
B. Secrete substances that attract prey
C. Secrete substances that repel predators
D. Facilitate cutaneous respiration

Q53

Insects have different mouthparts and for each type of feeding they are adapted in a specialized way. The 'primitive' arrangement of mouthparts is seen in the cockroach where they are used for chewing. When specialised food sources are exploited, the mouthparts are modified, sometimes considerably, so that the food may be ingested efficiently. Figure 1 shows different mouthpart types in insects and Figure 2 presents some insect species.

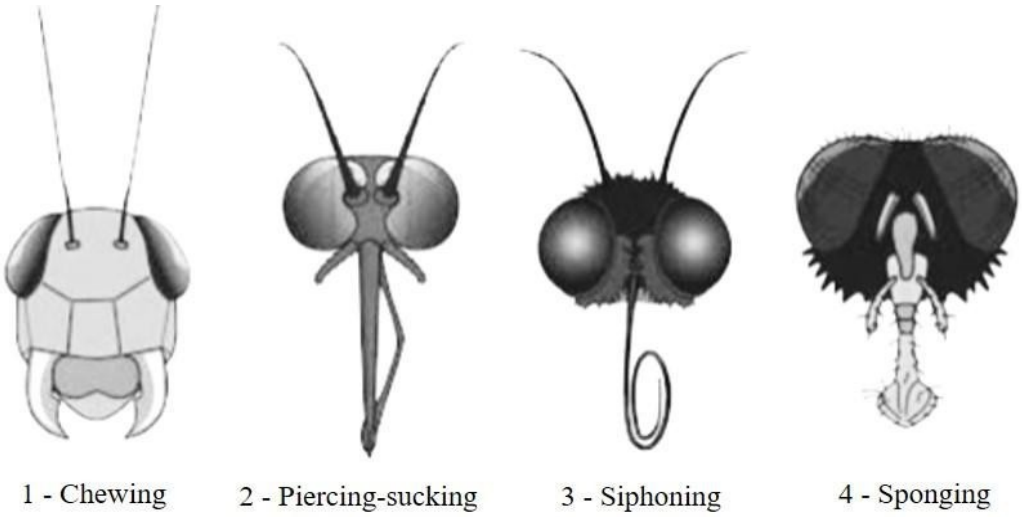


Figure 1. Four general types of mouthparts in insects.(insert nº with translation)

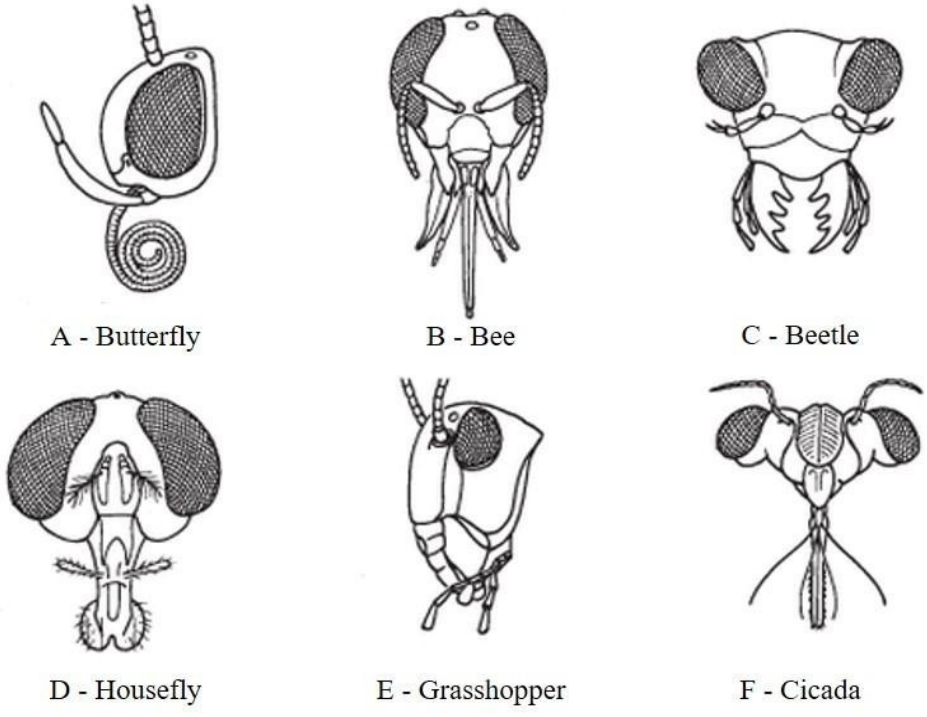


Figure 2. Mouthparts of six insect species. (insert Letter with translation)

Indicate whether each of the following statements is true (T) or false (F).
(*4 points, 1 point each correct answer*)

A. Species A is of the siphoning type, B is of the piercing-sucking type, D is of the chewing type, E of the piercing-sucking type
B. Species A is of the piercing-sucking type, B is of the siphoning type, C is of the chewing type, D is of the sponging type
C. Species A is of the siphoning type, C is of the chewing type, D is of the piercing- sucking type, E is of the sponging type
D. Species A is of the siphoning type, B is of the piercing-sucking type, C is of the chewing type, D is of the sponging type

Q54

Otoliths are acellular concretions of calcium carbonate and other inorganic salts, which develop over a protein matrix in the inner ear of vertebrates and are associated with balance and acoustic functions. In fish they record information in their microstructure and chemistry at different temporal scales related to their growth and environment. The largest of the three pairs of otoliths (sagitta) is the most studied presenting great morphological variability and being species specific.

Figure 1 presents the anatomical features of a fish sagitta otolith. **Table 1** presents five sagitta otolith descriptions. **Figure 2** presents sagitta otoliths from three species.

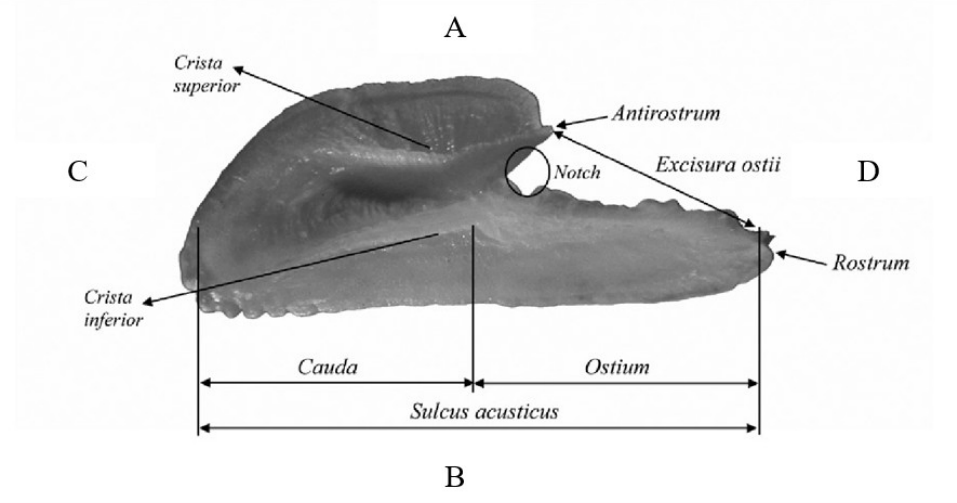
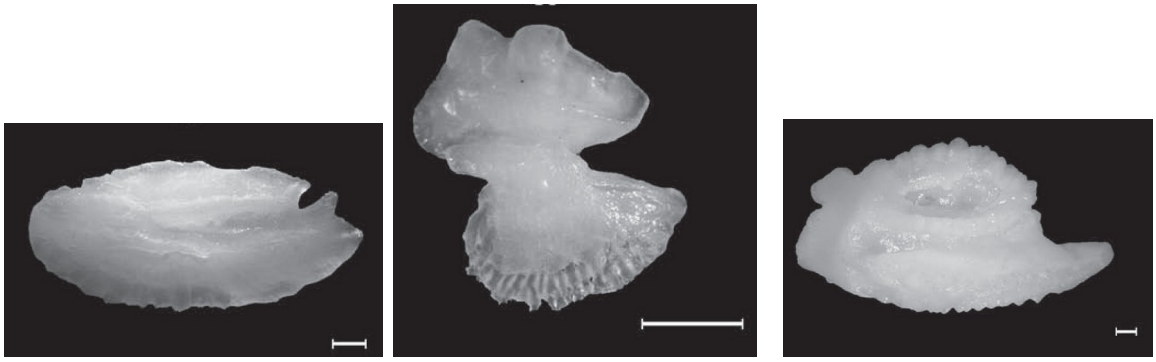


Figure 1. Anatomical features of a sagitta otolith used for morphological descriptions. A – Dorsal; B – Ventral; C – Posterior; D – Anterior.

Table 1. Otolith descriptions for five fish species. .

D1	**Forkbeard** (*Phycis phycis*) Shape: lanceolated to oblong, anterior region wider, margins crenate in the smaller otoliths. *Ostium* and *cauda*: undifferentiated. Anterior region: round to oblique-angled, *rostrum* poorly defined; *antirostrum* absent; *excisura ostii* very narrow without a notch. Posterior region: lanceolated to peaked.
D2	**Boar fish** (*Capros aper*) Shape: asymmetric with ventral and dorsal areas almost equally developed, ventral margin irregular. *Ostium*: funnel-like, shorter than the *cauda*. *Cauda*: tubular, straight, bordered by raised crests. Anterior region: double-peaked; *rostrum* and *antirostrum* large, broad and round or slightly pointed, *rostrum* longer; *excisura ostii* wide with an acute, deep notch. Posterior region: irregular to double-peaked.
D3	**Black scabbardfish** (*Aphanopus carbo*) Shape: elliptic to oblong, ventral margin sinuate to crenate. *Ostium*: funnel-like, shorter than the *cauda*. *Cauda*: tubular, straight, ending half way to the posterior margin. Anterior region: peaked; *rostrum* short, broad, pointed; *antirostrum* narrow, pointed; *excisura ostii* wide with or without a deep, acute notch. Posterior region: round.
D4	**Blue jack mackerel** (*Trachurus picturatus*) Shape: lanceolated, dorsal margin sinuate to irregular. *Ostium*: funnel-like, shorter than the *cauda*. *Cauda*: tubular, curved, strongly flexed posteriorly, ending close to the posterior-ventral margin. Anterior region: peaked; *rostrum* long, broad, very pointed; *antirostrum* short, broad, round; *excisura ostii* wide with a shallow notch. Posterior region: angled to oblique.
D5	**Bluemouth** (*Helicolenus dactylopterus*) Shape: elliptic. *Ostium*: funnel-like, longer than the *cauda*. *Cauda*: tubular, straight, wider posteriorly, ending far from the posterior margin. Anterior region: peaked; *rostrum* moderately long, broad, pointed, curved to the dorsal region; *antirostrum* absent; *excisura ostii* wide with a bite shaped notch in the larger otoliths. Posterior region: round to oblique.



A B C

Figure 2. Three different species (A, B and C) sagitta otoliths.

Indicate whether each of the following statements is true (T) or false (F).

(*4 points, 1 point each correct answer*)

A. Species A is *Aphanopus carbo*, B is *Capros aper* and C is *Phycis phycis*
B. Species A is *Trachurus picturatus*, B is *Capros aper* and C is *Phycis phycis*.
C. Species A is *Aphanopus carbo*, B is *Capros aper* and C is *Helicolenus dactylopterus*
D. Species A is *Trachurus picturatus*, B is *Aphanopus carbo* and C is *Phycis phycis*

CONGRATULATIONS!

YOU MADE IT TO THE END!